

Vol. 49, No. 1

March 1961

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

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CAMBRIDGE UNIVERSITY PRESS

Bentley House, 200 Euston Road, London, N.W. 1

American Branch: 32 East 57th Street, New York 22, N.Y.

Price Thirty Shillings net



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Host finding by aphids in the field

III. Visual attraction

By J. S. KENNEDY AND C. O. BOOTH

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(Received 12 April 1960)

SUMMARY

The spectral energy distribution of the light reflected from various leaves and soils has been measured and corrected for 'standard' sunlit conditions, and then compared on the basis of Moericke's evidence that aphids of many species distinguish two parts of the spectrum as colours, alighting and probing in response to wavelengths greater than 500 m μ .

Alightments by *Brevicoryne brassicae* and *Myzus persicae* in the field occurred preferentially on leaves reflecting a greater proportion of long-wave energy, with little or no regard for the 'botanical' (plant-taxonomic) host status of the plants for each aphid. The colour attraction of such 'yellow-sensitive' aphids serves rather to bias their alightments towards plants of the appropriate physiological type.

Laboratory experiments confirmed the existence, inferred from field observations, of a second, non-specific mechanism of visual attraction normally involved in alightments on plants, namely a close-range optomotor reaction with both orienting and arresting components.

The light reflected from various soils embodied maximum energy at the extreme long-wave end of the spectral range affecting aphids, while the leaf energy peak was 540-560 m μ . The predominantly long-wave emission from both leaves and soils contrasts sharply with the light from clear or clouded skies, wherein the peak energy region lies below 500 m μ . The aphids' type of colour vision thus provides for a primary discrimination between sky and ground, beside any subsidiary discriminations among plants and soils.

Low-flying *Aphis fabae* were attracted to white surfaces as well as to yellowing leaves in the field. The change in the migrant's behaviour from 'distance flight' in the upper air to low-level 'alighting flight' appears to be due, not to a reversal of the response to short-wave sky light from positive to negative, but simply to a relative strengthening of the positive response to long-wave light from the ground.

INTRODUCTION

Apart from olfactory attraction to plants, of which there is no evidence whatever (Kennedy, Booth & Kershaw, 1959*a, b*), two types of visual response have been suggested as responsible for the alightment of low-flying aphids on plants. The first is a directed colour reaction (phototaxis) of which Moericke (1952 *et seq.*) has provided abundant circumstantial evidence. The second is an optomotor reaction, alightment supposedly being provoked by objects 'looming up along the path of the flying insect' (Kennedy, 1950), but no evidence of this has yet been published. The present work confirms, extends and in some respects modifies Moericke's conclusions concerning colour attraction, with more measurements of the spectral composition of the light reflected from leaves and soils; and also provides evidence of optomotor attraction.

The only unequivocal experiments so far reported on colour vision in aphids are those of Moericke (1950), whose measure was the probing response of walking apterous and alate virginoparae of *Myzus persicae* (Sulzer). Using brief exposures both to monochromatic spectral lights thrown on to grey paper, and to Ostwald coloured papers in sunlight, he found that the aphids regularly stopped and probed the substrate when it reflected light of wavelengths greater than about $500\text{ m}\mu$: green, yellow and orange, with a maximum effect in the yellow. They walked on without stopping in shorter wavelengths—blue, violet and ultraviolet—but this region of the spectrum was *complementary* to the longer-wave region inasmuch as the aphids stopped and probed promptly on grey immediately after exposure to blue and violet, showing the phenomenon of successive colour contrast. The frequency of probing was independent of light intensity over a wide range, as was the position of the boundary ($500\text{ m}\mu$) between the two regions; and no probing occurred on passing from one shade of grey to another. Thus the aphids distinguished the two regions of the spectrum as true colours, and it was further shown that this was mediated by the compound eyes.

All subsequent published work on aphid colour vision has been concerned with the alighting response of flying aphids in the field, where the responsiveness and species composition of the available aphids, and likewise the quality and intensity of incident and reflected light, vary in an uncontrollable manner. Nevertheless, Moericke's (1952, 1953, 1955*a-c*) extensive experiments with water traps have established that flying alatae of many species of Aphididae distinguish phototactically the same two regions of the spectrum as do walking *M. persicae* in terms of probing. The field aphids made directed alightments in large numbers on green, yellow and orange surfaces, especially yellow (with and without ultraviolet and infrared filters), but rarely alighted on red, blue, violet, white, black and a series of greys.

Moericke (1952) early noted that the positive response to greens would serve the function of host finding in a very general way, but at that time saw no host-finding function in the still stronger response to yellow. When he compared alightments by *M. persicae*, *Aphis fabae* Scopoli and *Brevicoryne brassicae* L. on the leaves of various herbaceous plants exposed normally or submerged in water traps, none was as attractive as yellow paint or paper, and the leaves were alighted upon in the order of their apparent yellowness to our eyes regardless of their relative host status for each aphid

(Moericke, 1952, 1953, 1955*a*). However, further work with yellow water traps by Moericke (1955*a-c*), Eastop (1955), Heathcote (1957) and Lamb (1958) revealed differences between species in responsiveness to the trap yellow, some species being apparently quite unresponsive. It seemed that there might after all be an element of host specificity in the colour preferences of aphids (Moericke, 1955*a*). But the comparative measurements of host and non-host leaf colours required to test this hypothesis have been made in only one case, with disappointing results (*Hyalopterus pruni* (Geoffroy); Moericke, 1955*b*).

Moericke (1953, 1955*a*, p. 49) rejected the second, optomotor hypothesis of alighting because he found that uniformly yellow surfaces attracted more aphids than surfaces of the same size on which the yellow ground was interrupted by black or green stripes. This is confirmed for white surfaces in the present work. The number of alighters in these experiments was evidently governed primarily by the phototactic reaction proper, that is, by the total area rather than the form of the reflecting surface. But these results do not preclude the existence of an optomotor reaction which could play a larger part when the interrupted surface was less bright, like leaves. The attraction observed to bright yellow or white surfaces consists of a rapid, unhesitating, directed flight, often over several decimetres. On the other hand, when a flier arrives without prior response within a few centimetres of a leaf, it often hesitates, turns toward it and instead of going straight in to land, changes to a hovering type of flight close in front of the leaf, as if 'sniffing it', before alighting, or flying on (Kennedy *et al.* 1959*b*). It was to account for this different kind of orienting-arresting effect of leaves in the field that an optomotor effect was postulated (Kennedy, 1950). Such an optomotor reaction has been demonstrated by Ibbotson (unpublished) in walking apterae of *A. fabae* and Moericke (1955*a*, p. 72) describes fliers hesitating and hovering about in the air over the margin of a large yellow dish.

COLOUR COMPARISONS

Reflectance measurements and their application

These measurements, like those of Moericke (1955*a, b*), were of the diffuse (35–55° C.) reflexion from the given surfaces, as a percentage of that from a white standard surface exposed to light of the same wavelength. Moericke used a magnesium oxide standard in a Beckmann spectrophotometer, and we a fresh magnesium carbonate standard in a Unicam S.P. 500 spectrophotometer. In order to derive curves of reflected spectral energy distribution under natural daylight we have applied two corrections to Moericke's and our own measurements alike. One correction is for the incomplete reflectivity of the standard surfaces in the ultraviolet (Benford, Lloyd & Schwartz, 1948; Benford, Schwartz & Lloyd, 1948) shown in Fig. 1. The other is for the spectral energy distribution of the solar energy incident at the earth's surface. Moericke's uncorrected curves, like Lamb's (1958), exaggerate the amount of energy reflected below 400 m μ . in natural daylight, for there the incident energy decreases steeply to a negligible value at about 300 m μ owing to absorption by atmospheric ozone (compare the two curves in Fig. 2).

The 'standard' solar energy distribution curve of Moon (1940) has been used throughout for correction purposes (Fig. 1). It refers to the direct sunlight reaching sea-level through an atmosphere containing average quantities of water vapour, dust and ozone over Europe or the U.S.A. in spring and summer, with an optical air mass of 2 (air mass = $\sec \theta$, where θ = zenith angle of sun). The total energy between 300 and 640 $m\mu$ is then about 280 $W./m.^2$, and under such conditions the light from the rest of the sky contributes relatively little. When the sun is obscured by cloud the peak energy region is shifted toward the shorter wavelengths (Taylor & Kerr, 1941). When the sun declines and the optical air mass increases there is a well-known shift in the opposite direction (Seybold, 1936; Moon, 1940). Such natural variations in the shape of the incident energy curve will alter the degree but not the sense in which the re-emission curves of surfaces differ.

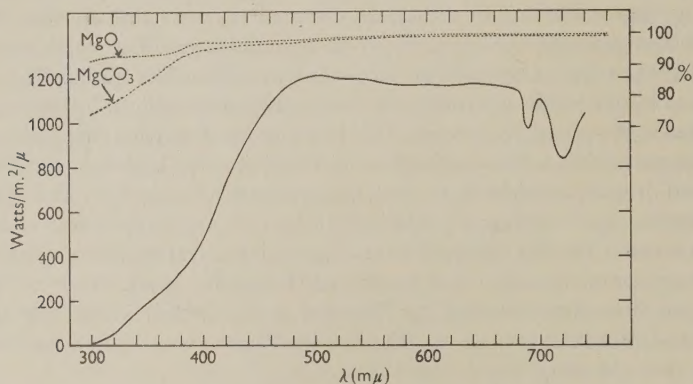


Fig. 1. Broken lines above: percentage reflectance curves of standard white surfaces used in spectrophotometers, after Benford *et al.* (1948). Continuous line: spectral energy distribution of sunlight as integrated by Moon (1940) under certain conditions specified in the text, and used as a standard reference curve throughout this paper.

These measurements refer exclusively to diffuse reflexion and take no account of direct superficial reflexions. The latter vary greatly according to surface structure and will affect the quality as well as the intensity of the reflected light received by flying aphids approaching from appropriate angles. Direct sky reflexions have a 'whitening' effect on reflected spectral energy distributions and reduce the colour contrast between any surface and the sky. On the other hand, flying aphids will often see leaves by transmitted light, as Moericke (1943, 1955*a*), pointed out, and then the intrinsic leaf colours will not be appreciably diluted either by direct or by diffuse surface reflexions.

On the basis of Moericke's work mentioned in the Introduction, we have used the following method to express the reflectance measurements in terms likely to be relevant to the behaviour of low-flying aphids. Wavelengths greater than 640 $m\mu$, to which he found the aphids were very insensitive, have been disregarded. This leaves an effective range from 300 to 640 $m\mu$ in the field, separable into the two regions distinguished as

colours by the aphids: green-yellow-orange, above 500 m μ ; and blue-violet-ultra-violet, below 500 m μ . We assume, following Moericke for the time being, that the first, long-wave region tends to elicit directed alightment by many aphids when they are flying low and ready to alight in the field, including the species we are concerned with below; while the second, short-wave region tends rather to turn them away.

Table 1. *Summary of measurements of the spectral energy distribution of light of wavelengths between 300 and 640 m μ reflected from various surfaces*

Total energies calculated for sunlit conditions as specified in Fig. 1 and the text, together with the energy ratios of aphidological interest explained in the text.

Source	Particulars	Total energy W./m. ² of surface	'Long/short' energy ratio	Relative energy curve shown in Figs. 3-9
Leaves (mature unless otherwise stated)				
Sugar beet	After Moericke (1955 <i>b</i>)	25.7	3.19	—
	July 1959 (dark)	23.1	2.92	—
	July 1959 (medium)	24.2	3.22	3, 5
	July 1959 (pale)	29.2	3.14	—
	Sept. 1959 (dark)	22.2	3.10	—
	Sept. 1959 (medium)	21.6	3.34	—
	Sept. 1959 (pale)	21.1	3.77	—
	Oct. 1958	23.3	2.42	—
	July 1959 turgid	22.7	2.84	6
	July 1959 wilted (same leaf)	21.4	5.62	6
Cabbage	July 1959 (dark)	27.6	1.99	—
	July 1959 (medium)	29.6	2.07	3
	July 1959 (pale)	45.0	2.45	—
	Sept. 1959 (dark)	30.4	1.59	—
	Sept. 1959 (medium)	22.1	2.03	—
	Sept. 1959 (pale)	28.9	2.18	—
	Oct. 1958	19.7	2.42	—
Brussels sprout	Oct. 1958 (lower surface)	40.3	2.23	4
	Oct. 1958 (upper surface)	19.2	2.02	—
Peach	Oct. 1958, mature (<i>M</i>)	17.5	2.35	5
	Oct. 1958, <i>MO</i>	20.1	2.44	4
	Oct. 1958, <i>O_a</i>	34.4	4.12	4
Spindle	Oct. 1958, mature (<i>M</i>)	14.5	2.31	5
	Oct. 1958, <i>MO</i>	27.4	3.79	—
	Oct. 1958, <i>O_a</i>	43.2	5.65	—
Potato	After Moericke (1955 <i>b</i>)	26.9	2.71	5
Phragmites	After Moericke (1955 <i>b</i>)	22.9	2.65	—
Soils				
Yellow sandy	July 1959	31.0	2.98	7
Light loam	July 1959	24.0	2.48	7
Light loam wet	July 1959	13.4	2.13	7
Black fen soil	July 1959	10.0	1.82	7
Light loam	After Moericke (1955 <i>b</i>)	43.8	2.76	—
Tarmac (worn)	July 1959	29.2	1.69	3

As a measure of the expected resultant effect on such aphids of light of a given spectral energy composition we have therefore taken the ratio:

$$\frac{\text{energy between } 500 \text{ and } 640 \text{ m}\mu}{\text{energy between } 300 \text{ and } 500 \text{ m}\mu}$$

This 'long/short ratio' has been calculated for all the light sources here considered from measurements of the areas under the two stated portions of each spectral energy distribution curve. These ratios are assembled in Table 1 together with the total energies between 300 and 640 m μ and will be discussed below in the context of the aphid observations.

Moericke (1955*a*, *b*) believed that a certain amount of ultraviolet within a predominantly yellow or green emission might to some extent help, rather than hinder, the alightment of aphids in general, if less strongly than in *Hyalopectus pruni*. Variation

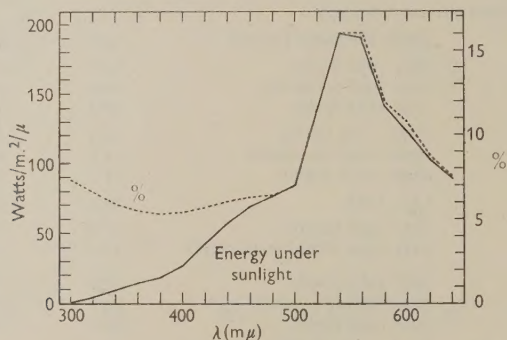


Fig. 2. Broken line: uncorrected percentage spectral reflectance curve from a potato leaf after Moericke (1955*b*, fig. 14). Continuous line: calculated spectral energy distribution of light reflected from the same potato leaf under direct sunlight having the spectral energy distribution shown in Fig. 1.

between species in this respect might partly explain the above-mentioned ineffectiveness of yellow traps for some species. However, our present species are not among those; and the ultraviolet emission is a small fraction of the total from leaves in daylight. Omitting this fraction from the calculation of the ratios, or even transferring it from the denominator to the numerator, does not alter the sense in which the ratios differ. The effect of the aphids, on the other hand, might of course be altered if they were especially sensitive to ultraviolet. The ratios are strictly provisional and relative measures of the expected effect of the light sources on flying aphids and call for correction by measurements of the relative sensitivity of each aphid species, form and stage, to light of different wavelengths.

In addition to the calculated ratios in Table 1, a representative series of reflectance curves is reproduced graphically in Figs. 2-9. Only one sample curve (Fig. 2, continuous line) is given in absolute units corrected as described above, because a given surface

may reflect more light than another at all wavelengths, both above and below $500\text{ m}\mu$, and it is then difficult to compare the two curves by eye with respect to the proportions of their total energy emitted above and below $500\text{ m}\mu$, i.e. as colours for an aphid. The remaining reflectance curves reproduced in this paper (Figs. 3-9) therefore show not absolute but relative energies, all the curves being adjusted to equal total energy (area under the curve) as was done by Taylor & Kerr (1941) with their sky curves. The actual total energy is given against each curve and the absolute curve can be derived by multiplying the relative values on the vertical axis of each graph by this total energy figure.

RESULTS

Cabbage and sugar beet leaves in summer: alighting by Brevicoryne brassicae (L.)

Although cabbage (*Brassica oleracea* L.) is a favoured host plant of *B. brassicae*, while sugar beet (*Beta vulgaris* L.) is not one of its hosts, direct observation of flying *B. brassicae* in late August 1952 suggested that they were alighting on beet leaves more often than on cabbage (Kennedy *et al.* 1959*b*). To check this, twenty 10 cm. Petri dishes containing water with a trace of detergent were laid out in four rows of five, 30 cm. apart, on a tarmac drive from 12.30 to 17.00 hr on 2 September. Ten of the dishes rested on 9.5 cm. discs of mature cabbage leaf and the other ten, alternating within and between rows, rested on discs of mature sugar-beet leaf. The twenty dishes were set out a second time from the morning of 3 September until midday on 4 September, similarly arranged but now with the addition of three interpolated rows each of four plain water dishes resting directly on the tarmac. The trapped aphids were counted and preserved at the end of each exposure and subsequent examination showed 99% to be *B. brassicae* females (Kennedy *et al.* 1959*b*). The dishes over sugar-beet leaf discs caught twice as many of these alatae as the dishes over cabbage discs in both exposures ($P < 0.02$ and < 0.001 , respectively), while the cabbage dishes caught no more than the plain tarmac dishes (Table 2).

Table 2. *Results of exposing petri dishes of water overlying leaf discs or tarmac to flying Brevicoryne brassicae outdoors*

	Mean catch per dish		
	Sugar beet (10 dishes)	Cabbage (10 dishes)	Tarmac (12 dishes)
First exposure	53.9 $\pm$ 33.7	22.2 $\pm$ 14.4	—
Second exposure	22.2 $\pm$ 8.3	10.6 $\pm$ 4.2	10.1 $\pm$ 3.1

Pot plants were later grown again in the glasshouse to the same stage as those used in the 1952 experiments, to provide mature leaves for the reflectance measurements made on the dates shown in Table 1. Fig. 3 shows the relative energy curves derived from measurements in July 1959, together with the curve from a sample of tarmac with the black tar worn in places to expose the grey surface of the granite chippings below, like the tarmac on which the dishes had rested. Fig. 3 refers to the naked leaves and tarmac to make these curves comparable with others, although in these experiments

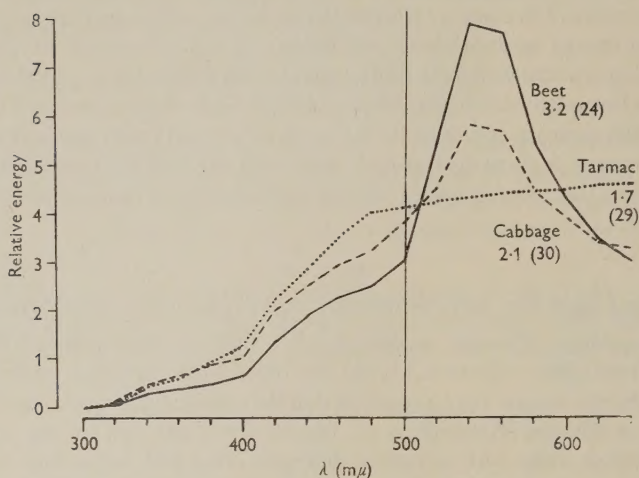


Fig. 3. Relative energy curves for light reflected from the upper surfaces of mature leaves of sugar beet and cabbage in July, and from worn tarmac, under direct sunlight having the spectral energy distribution shown in Fig. 1. The open figure against each curve is the ratio between the areas under the curve to the right and left of the dividing line at $500\text{ m}\mu$ (the 'long/short' ratio). The figure in brackets is the total energy between 300 and $640\text{ m}\mu$ in W./m^2 ; multiplying the arbitrary relative energy values by this figure converts the curve to absolute units.

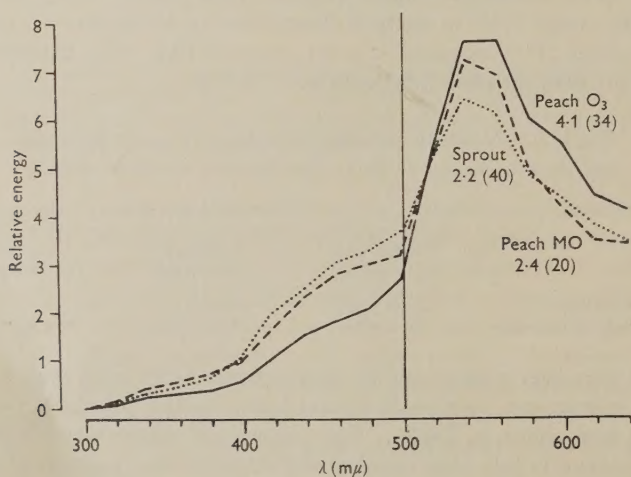


Fig. 4. Reflectance curves as in Fig. 3, from the upper surfaces of peach leaves in early (MO) and late (O_3) senescence, and from the lower surface of a mature brussels sprout leaf, in October.

the light received by the aphids had passed twice through the water and glass of the dishes. Absorption by this thickness of water can be neglected (about 2% where it is greatest, at 300 m μ). The amount of absorption by the glass dishes actually used is not known; in the extreme case of a very strongly absorbing glass ('G 2' in the *International Critical Tables*, 5, 271 (1929)) of twice the dish thickness, all the incident light would have been absorbed between 300 and 340 m μ , about 90% at 360, 25% at 380, 7% at 400 and a negligible percentage from 500 m μ upwards. Thus Fig. 3 exaggerates the proportion of ultraviolet reflected from all three surfaces. For comparative purposes, however, this makes little difference and it has been ignored in Fig. 3 and Table 1.

The July and September measurements always showed larger 'long short' ratios for beet than for cabbage (Table 1) as expected from the white waxy 'bloom' on the cabbage leaves. The cabbage ratios tended to be rather higher than the tarmac ratios, but here, where the aphids showed no discrimination, the difference is small. There was a consistently higher proportion of ultraviolet reflected by the cabbage leaves compared with the beet. Even if we assume with Moericke that such admixed ultraviolet does not oppose but favours alightment, any advantage possessed by the cabbage leaves in this respect was apparently outweighed by their reflecting more blue and violet as well.

*Peach and brussels sprout leaves in autumn: alighting by
Myzus persicae gynoparae and Brevicoryne brassicae*

During the massive autumn migration of *M. persicae* in 1947, a majority of the flying aphids that were observed to approach within about 30 cm. of any leaf, turned aside without alighting. When they were observed approaching peach (*Prunus persica* L.) and brussels sprout (*Brassica oleracea* L.) plants of about the same size standing side by side, a significantly higher proportion turned away from the sprout leaves with the result that three times as many alighted on the peach (Kennedy *et al.* 1959*a*). Two other surfaces seemed to divert close approachers about as often as the sprout leaves: light grey brick walls, and colourless shiny sticky traps. These three types of surface all appeared to the human eye whiter than the senescent leaves of the peach and spindle trees on which the fliers alighted more often. The reflectances of sprout, peach and spindle leaves from outdoor plants were measured at the same time of year in 1958. Then, as in 1947, most of the peach and spindle leaves were visibly senescing and had reached a stage between MO and O_4 (Kennedy, Ibbotson & Booth, 1950), while the sprout leaves were large mature ones mostly upturned to form a 'head' with their lower surface presented to the approaching aphids. Fig. 4 shows that the sprout leaves reflected more in the blue-violet-ultraviolet relative to longer wavelengths than the senescent peach leaves, thus yielding smaller long/short ratios which are very similar to those of the cabbage leaves above (Table 1).

The gynoparae of *M. persicae* thus resembled the virginoparae of *B. brassicae* in alighting preferentially on leaves with larger long/short ratios, regardless of their host status. At the same time there were seven *brassicae* among the twenty-two aphids caught alighting on the sprouts during these observations, and no *brassicae* at all among

the eighty-eight aphids caught alighting concurrently on peach (Kennedy *et al.* 1959*a*). The overwhelming majority of fliers were *M. persicae* at the time and chances of catching any *brassicae* were extremely small except on the sprouts which most *persicae* avoided, so it is quite possible that *brassicae*, too, was alighting preferentially on peach. But the results suggest it at any rate did not avoid alighting on the sprouts as often as *persicae*, showing in effect a weak host-specific bias, which would be consistent with Moericke's (1955*b*, p. 66) finding that *B. brassicae*, although caught in smaller numbers in water traps containing cabbage leaves than in those containing lettuce and beet leaves, nevertheless entered the cabbage dishes more often than *persicae* and other species.

Woody and herbaceous plants at different seasons

Peach and spindle are primary hosts of *M. persicae* and *A. fabae* respectively, and the senescent leaves they bear in the autumn when they are re-colonized by the aphids gave long/short ratios that were not only greater than those of the mature brassica leaves, but greater also than those of mature beet leaves measured at that time. By

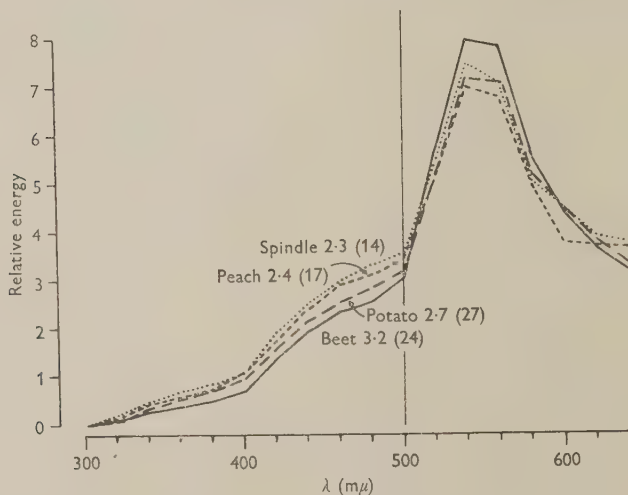


Fig. 5. Reflectance curves, as in Fig. 3, from the upper surfaces of mature leaves of two woody and two herbaceous plants.

October the beet leaves were passing into their duller green overwintering condition and their ratios had fallen as low as those of the brassicas (Table 1). At that time leaf growth and senescence is ceasing on such overwintering herbaceous plants and the majority of their leaves become less yellow than the senescing foliage of trees, thus presumably increasing the relative attractiveness of the trees (if we disregard the effects of disease in sugar beet root crops).

The other measurements suggest that this situation will be reversed during the spring and summer migrations of the same aphids, when they colonize their secondary,

herbaceous hosts. Fig. 5 and Table 1 show that even the mature leaves of sugar beet (July–September) and potato give somewhat greater long/short ratios than mature leaves of peach and spindle. It follows that such herbs will be considerably more attractive than the trees in the summer, because at that time the trees bear mainly mature leaves whereas the herbs are growing, and in varying degree also senescing. Moericke (1955*a*, p. 57) found that young growing leaves, as expected from their fresh, yellowish appearance, attracted many more aphids than mature leaves of the same plant (maple), and that yellows-diseased beet leaves attracted more than healthy ones. It seems safe to assume that senescent leaves are also more attractive than mature ones of the same plant, judging by their yellower appearance and the measurements of peach and spindle leaves in Table 1 and Fig. 4.

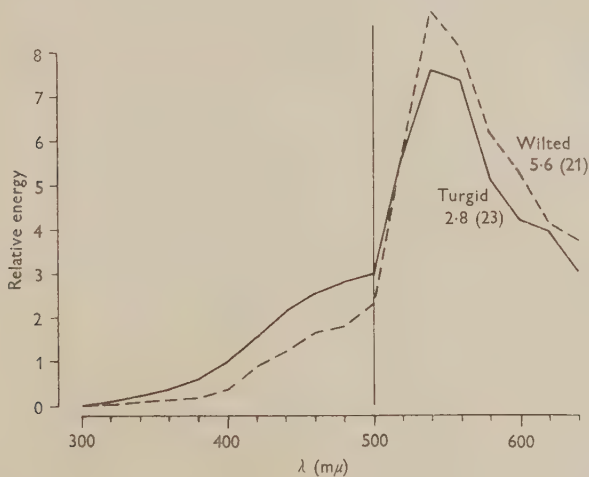


Fig. 6. Reflectance curves, as in Fig. 3, from the upper surface of a mature sugar-beet leaf before and after wilting.

Water strain in the summer should enhance the attractiveness of herbs relative to trees. Fig. 6 illustrates quantitatively what is visible to the eye, that wilting of a mature sugar beet leaf is accompanied by an increase in the long/short ratio. Beets and other herbs wilt readily during the day under water strain in the field, recovering every night so long as the strain is not too severe (Kennedy & Booth 1959). Thus they exhibit a diurnal colour cycle, such that they are most attractively yellow when low-flying aphids are most likely to be there to see them, in the late morning, afternoon and early evening. Spindle and other tree leaves do not wilt visibly under water strain in the field (Kennedy & Booth, 1958, 1959) nor do they exhibit a noticeable diurnal green-yellow cycle. And continued water strain leads to some paling of spindle leaves but not to the progressively yellowing senescence induced in beet leaves by the same field conditions (Booth & Kennedy, unpublished).

Leaves, soils and skies

The relative energy curves from three Cambridgeshire soils are shown in Fig. 7. The yellow sandy soil and the black fen soil were chosen as colour extremes to the human eye, most soils appearing more like the light loam. Moericke's (1955*b*, fig. 15)

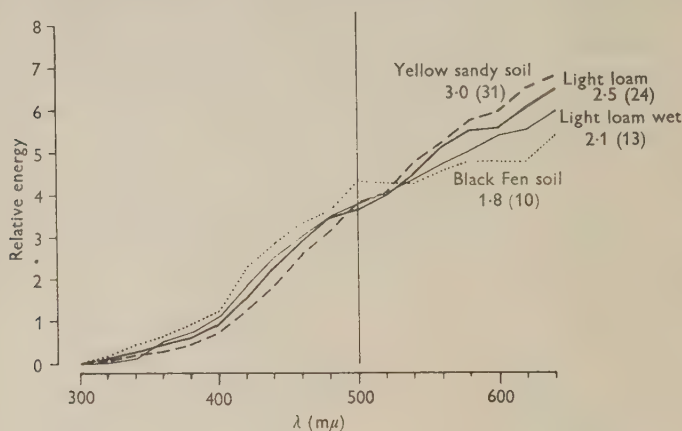


Fig. 7. Reflectance curves, as in Fig. 3, from three soils in a dry condition and one of the same when moistened.

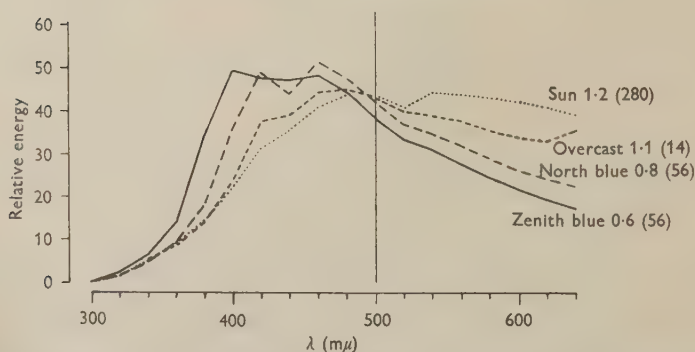


Fig. 8. Relative energy distribution curves of direct sunlight and three types of sky, re-calculated as explained in the text after Taylor & Kerr (1941). Figures against curves, as in Fig. 3. Total energies (in brackets) from blue skies taken as 20 % of direct sunlight, and from cloud overcast as 5 %.

curve for a light loam, when corrected and converted in the same way, lies roughly between the light loam and yellow sandy soil curves in Fig. 7. The distinctive feature of all the soil curves is their more or less steady rise to $640\text{m}\mu$ (and beyond). But because the peak energy region for all the leaves is also above $500\text{m}\mu$, in the green-

yellow at 540–560 $m\mu$, the leaf and dry soil ratios are not very different, nor are their total intensities (Table 1). The exceptionally yellow soil gives a ratio as great as the beet leaves. The more normal loams give ratios that are smaller than most from the beet leaves. But these ratios are close to those from Moericke's potato and *Phragmites* leaves and larger than most from our brassica leaves. This presumably explains why the aphids in Moericke's (1953, fig. 6) water-trap experiment showed rather a weak preference for alighting on cabbage and potato leaves before soil (cf. the tarmac experiment above). Moistening a soil, however, heightens the contrast with leaves by reducing the soil colour ratio as well as the total energy reflected (Fig. 7 and Table 1). The preference for leaves before soil may often owe something to the fact that leaves reflect more in the most stimulating, yellow part of the spectrum above 500 $m\mu$, while the soils reflect more in the less stimulating, orange part of it.

Moericke (1953, 1955 *a-c*) reported that when plants or yellow traps were surrounded by white or blue material, or by a cloth resembling the soil except that it reflected more below 440 $m\mu$ (violet-ultraviolet), alightments were drastically reduced. Many fliers turned away, upwards or horizontally, on encountering patches of ground thus coloured to give small long/short ratios. The light reflected from the ground, which has a large long/short ratio alike when the soil is bare and when it is blanketed with plants, is thus a factor in maintaining that more or less horizontal, low-level flight over and among plants which Moericke called *Befallsflug* ('attacking flight'), a term we may translate as 'alighting flight'.

The aphid engaged in alighting flight is exposed at the same time to the incident light from the sky, without which low-flying virtually ceases (Broadbent, 1949; Johnston, Taylor & Haine, 1957). Fig. 8 gives the spectral compositions of light from various skies, re-calculated for equal energies between 300 and 640 $m\mu$ from Taylor & Kerr's (1941) relative energy curves which were based on equal energies between 400 and 700 $m\mu$. Values between 300 and 400 $m\mu$ have been estimated from extrapolations of their curves from 400 down to zero energy at 300 $m\mu$. It is plain from Table 1, and from comparison of Fig. 8 with the leaf and soil curves preceding, that the colour contrast between all the skies on the one hand, and all the leaves and soils on the other, is much greater than any differences among the leaves and soils. Unlike the leaf and soil curves, the three sky curves all have their peak energy regions below the critical wavelength of 500 $m\mu$ and give correspondingly small long/short ratios. Thus aphids engaged in alighting flight are exposed continuously to a marked contrast between the light incident from above with its small long/short ratio and that reflected from below with its large one. This is their complete 'steady-state' stimulation, departures from which produce the observed positive or negative responses to ground objects.

White, black and interrupted surfaces: alighting by Aphis fabae Scop. gynoparae

During the massive autumn migration of *A. fabae* (*sensu lato*) in 1957 when alightments were being recorded on peach and spindle trees (Kennedy *et al.* 1959*b*), three paper cylinders 45 cm. high and 20 cm. in diameter were set out 23 cm. apart in a row on a table 76 cm. high, and alighters on them were collected continuously for 3 hr.

around midday on 8 October. One cylinder was matt black, another white Bristol board and the third made up of alternate 5 cm. stripes of the same black and white papers; the positions of the cylinders were interchanged every 30 min. In all, 213 alighters were collected, comprising 206 gynoparae and 7 males of *A. fabae* (*sensu lato*) only. Their incidence on the cylinders differed in the same way in all of the twelve 15 min. intervals of the exposure; most alighted on the white (total 119), fewer on the striped (72) and fewest on the black cylinder (22). This was because the all-white cylinder attracted the majority of the fliers that arrived in front of it or within about 30 cm. on either side, while they were attracted only to individual white stripes on the striped cylinder, turning to one of them from not more than 10 cm. away; and most fliers arriving in front of the black cylinder turned aside. Whether some fliers turned away from the white cylinder was not noticed.

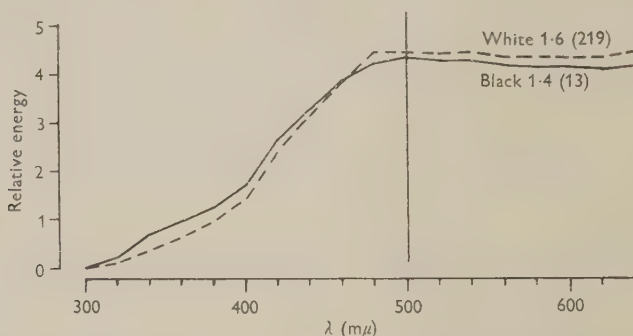


Fig. 9. Reflectance curves, as in Fig. 3, from the white (Bristol board) and black papers used in experiments with *A. fabae*. To convert the white paper curve to absolute units, the relative energy values must be multiplied by ten.

Fig. 9 shows that the white and black papers differed so little in reflected spectral energy distribution that the aphids' discrimination is probably attributable to the far greater total energy reflected from the white. The vegetation background was darker than the white paper and lighter than the black, so that the aphids were in effect turning from darker toward lighter frontal illumination in their observed responses at all three cylinders. At the same time, some 70% of fliers that approached within 10–15 cm. of the senescing leaves of nearby peach and spindle trees alighted on them as well (Kennedy *et al.* 1959*b*), although they reflected a much smaller energy total than the white paper (Table 1).

OPTOMOTOR ATTRACTION

The above field experiment with a striped paper cylinder gave no quantitative evidence of the postulated optomotor reaction, but this may have been merely because the phototactic reaction to white was predominant in these conditions, as pointed out in the Introduction. In an attempt to display the suspected optomotor responses

uncomplicated by phototaxis, vertical strips of black paper 4 cm. wide were exposed outdoors without any reflecting surface between them; simply spaced 4 cm. apart with the general background visible through the gaps. A wire frame 60 by 60 cm. carrying eight such strips was suspended with its base 90 cm. above the ground, alongside a similar frame carrying a continuous sheet of black paper and third frame entirely empty. The behaviour of fliers approaching the three frames was observed for 3 hr. on 14 October 1958, when moderate numbers of unidentified aphids were flying about the garden. Approximately forty alatae approached each frame in that time. All flew straight through the empty one. All but three turned aside when close to the solid one and flew round or over it, the other three alighting on it without prior hesitation. Among the approachers to the barred frame all but five hesitated and hovered a few cm. in front of one or more strips for several seconds, six of these alighting on a strip and the rest flying on through a gap; the other five approachers passed unhesitatingly through a gap.

Laboratory experiments designed to separate phototactic from optomotor reactions were carried out in an earlier model of the 'flight chamber' mentioned by Kennedy (1958), using laboratory-bred virginoparous alate *A. fabae* taken from the roof and walls of the culture cabinet (Kennedy & Booth, 1954) where they had already had some flight exercise. This apparatus (Fig. 10), unlike subsequent models, was installed in a dark room at 20° C. and had walls of black mosquito netting hanging straight down from the margins of the bolting silk screen (65 × 50 cm.) under the lights. Aphids flying up towards the lights were prevented from reaching the screen by the draught from the fan above the lights, but sooner or later they hit the side curtains and alighted there. By releasing about a hundred alatae below the screen and adjusting the fan speed, it was possible to create a dense 'swarm' of flying aphids in the form of a swirling column 15–20 cm. wide and about 40 cm. deep under the centre of the screen. The number of aphids participating in this swarm at any one time dwindled owing to alightments and lengthening stays on the curtains, until very few were flying after about an hour.

When the entire 65 cm. width of the rear curtain was covered with black paper from screen level down to 40 cm., there was no change in the behaviour of the fliers. When white paper (Bristol board) was substituted for the black, the column of fliers shifted *en bloc* towards that side of the chamber and dissolved in a matter of minutes owing to a succession of fliers being attracted to and alighting on the white surface. Evidently these fliers (like the *fabae* gynoparae attracted to the white cylinder in the field, above), were more sensitive phototactically to light received from the side than from above, since the top light was of course more intense than the reflected light for the paper. Even small reflecting surfaces, a few cm. across, held vertically inside the curtain, attracted fliers across the beam from the top lights, and routine use was made of this fact in subsequent experiments in order to obtain alightment on single leaves or other objects whenever the experimenter wished to terminate a flight. Only aphids that were very excited locomotorily, as during the first minute or so after the first take-off, or after the alighting response had been inhibited by repeated alightments on a non-host surface (Kennedy, 1958), failed to show this overriding directed response to

lateral illumination. Indeed, it was possible to maintain aphids continuously in flight up to the point of exhaustion only if the surroundings were entirely black and the experimenter wore a black gown and black gloves and kept his face up in the shadow above the screen level.

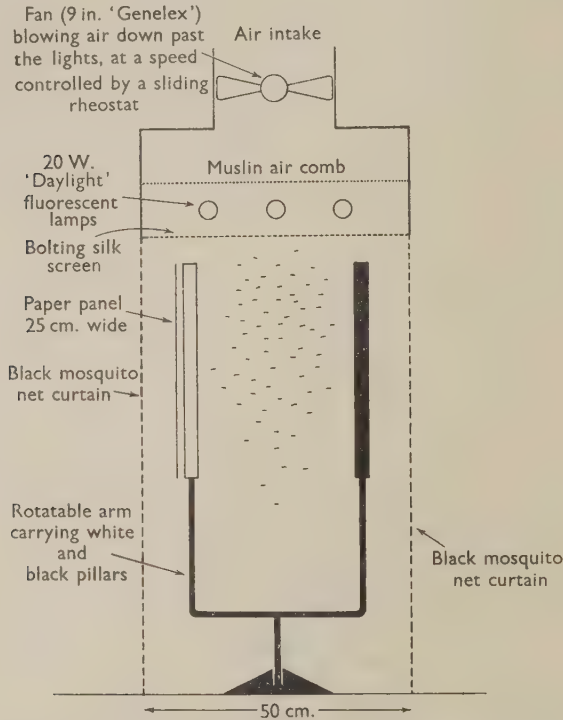


Fig. 10. Diagrammatic vertical section through the apparatus for maintaining a 'swarm' of flying aphids (indicated by dashes) for experiments on optomotor attraction. The observer stands on the right outside the mosquito net curtain and records alightments on the opposite pillar immediately in front of the paper panel.

For the experiments on optomotor attraction, a white paper panel 25 cm. wide and 40 cm. long was placed vertically in the centre of the rear curtain. This attracted fliers less frequently than the full-width white sheet so that swarms thinned out more slowly and could be used for alightment counts for 30-40 min. Two paper pillars (tubes) 4 cm. in diameter and 50 cm. long, one black and one white, were mounted vertically 30 cm. apart on a rotatable arm as shown in Fig. 10. Either one could be swung into position before the centre of the rear curtain away from the observer for alightment counts, while the other pillar stood unobserved inside the front curtain. Alightments on the rear pillar by fliers from a swarm were counted continuously for 1 min., the

pillar positions were then reversed, the aphids on them jolted off into flight again, counts continued for another minute on the new rear pillar, and so on. Three sets of such observations were made with the 25 cm. white panel behind the rear pillar, using a fresh swarm for each set. Two separate, control sets of observations were interpolated, again on fresh swarms, but without the white panel so that the background of the observed pillar was black. Many more aphids alighted on the white panel itself than on the same area when it was left black, but these were not counted.

Table 3. *Results of flight chamber experiments on optomotor attraction of Aphis fabae*

Background	Expt.	Total counts on each pillar	Mean no. alightments per count on each pillar		Comparison of successive counts		
			White	Black	White > Black	Black > White	White = Black
Black	2	10	13.1	2.6	18	1	0
	4	5	29.8	5.0	9	0	0
	Combined percentages				96	4	0
White	1	21	2.9	5.1	6	31	4
	3	10	3.8	7.0	2	16	1
	5	11	9.7	12.5	1	20	0
Combined percentages					11	83	6

The results in terms of alightments on the pillars are given in Table 3. Owing to the changing number of fliers available to alight as the swarm dwindled in the course of one set of counts, the table shows the frequencies at which successive counts on the black and white pillars differed in one sense or the other, as well as the mean absolute numbers alighting per minute count. In the control experiments with the pillars against a black background, there were several times more alighters on the white pillar than on the black, as expected. But with the white panel in place behind the pillar, there were more alighters on the black pillar than on the white. This result was inexplicable in terms of phototaxis alone and attributable to the optomotor stimulus of the black pillar against the white background, a stimulus that was virtually absent as far as the pillar was concerned, when it and its background were both white.

Table 3 shows that this optomotor effect brought about fewer alightments than the simple phototaxis, and the main reason for this was the smaller range over which the optomotor stimulus acted. The aphids were seen to fly directly and unhesitatingly toward the white pillar from 20 cm. or more away, but turned to the black pillar against a white ground from at most 10 cm. away. They then hesitated and hovered a few cm. before the black pillar as if 'sniffing it', sometimes for many seconds, and eventually alighted on it or turned away to rejoin the central swarm or alight on the white panel. During this hovering phase the down-draught carried them slowly down the length of the pillar showing that their rate of climb toward the top lights had diminished.

DISCUSSION

The reaction that brings a flying aphid into contact with a solid surface, and so makes it alight, is evidently not simple. The orientation component can be phototactic or optomotor or both, and the optomotor reaction involves a kinetic (arresting) component as well. The phototactic reaction is most strongly elicited by long-wave light, according to Moericke (1952 *et seq.*). The present evidence from leaf preferences is in general agreement with this, but the attraction of *A. fabiae* to upright white surfaces seems to conflict with it and especially with Moericke's (1955*c*) substantial evidence of repulsion by white. In fact this is not the first evidence that low fliers can sometimes be attracted to white: Folsom (1927), Moore (1935, 1937), Broadbent (1948) and Moericke (1952, fig. 1) have also recorded more alighters on white than on black.

Moericke (1955*a*, p. 63) stressed that by no means all approaching fliers respond either positively to yellow or negatively to white. He suggested that there might be two sorts of fliers at any one moment: 'alighters' responding positively to yellow and negatively to white, and 'non-alighters' not responding to either. In the present observations on *A. fabae*, however, a large majority of fliers responded positively both to white paper and to yellowish leaves. Some of them were evidently positive simultaneously to white light of moderate intensity and to dimmer light with a large long/short ratio.

The facts can be reconciled by a modification of Moericke's (1955*c*) hypothesis concerning the role of light reactions in the change-over from dispersal on the upper air to low-level host-finding flight. Fresh aphids taking off for the first time show a strong positive phototaxis which in the field takes them up into the sky (Moericke's 'distance flight') and in the laboratory can equally draw them to a light at the side or below (Moericke, 1955*a*, p. 33). An eventual reversal of this response to the shorter-wave part only of the spectrum was inferred by Moericke (1955*c*) from the negative reaction to white, blue or ultraviolet reflected from the ground, shown by presumably long-flown low fliers in the field. Moericke (1955*c*) therefore put forward the hypothesis that the development of a negative response to short-wave light might be what terminated the phase of 'distance flight' by driving fliers down from the sky again, and ushered in the next phase of low-level 'alighting flight' when the fliers are attracted only to predominantly long-wave light. In this unqualified form the hypothesis is inconsistent, not only with the positive response to white surfaces observed in a varying proportion of fliers, but also with the occurrence of low-level 'alighting flight' itself. Fliers responding negatively to the light incident from above and positively to that reflected from below could be expected only to descend, not to fly along more or less horizontally.

The inference is that aphids engaged in 'alighting flight' have not ceased to respond positively to short-wave light; and are kept in the air, yet close the ground, as a result of an uneasy balance between the upward pull of the sky light and the downward pull of the dimmer but predominantly long-wave light from plants and soil. The observed upward or sideways 'repulsion' of low fliers from short-wave reflecting surfaces on the ground does not warrant the conclusion that the fliers have now become negative

to such light, for in turning upward they are turning toward a brighter source of similar light and in turning sideways they are turning toward the long-wave light from the surrounding plants or soil. A strengthening of their positive response to the latter, long-wave light, relative to short, is the only change of responsiveness indicated by the evidence so far. The variable responsiveness of low fliers in the field is attributable to their being at different stages in this process, which is in any case presumably reversible like the other behavioural changes induced by flight (Moericke, 1955*a*; Johnson, 1958; Kennedy, 1958).

Positive phototactic responses to white and other colours with small long/short ratios will seldom end in alightment in nature where surfaces are seldom so coloured; such responses will usually be to the sky. Thus the *fabae* that turned toward upright white paper (and away from black) were behaving in the way that normally takes them through gaps between plants. Movement between and over foliage masses is the typical behaviour of low-flying aphids: that is what keeps them flying. Only a certain proportion, sometimes a small minority, of those approaching a plant, do not dodge but proceed to alight on it (Kennedy *et al.* 1959*a, b*). Indeed actual alighting on a plant requires an immediate change in phototactic colour responsiveness like the longer-term change discussed above, inasmuch as it means that the flier is now accepting a progressive displacement of the sky light in its eyes by the dimmer long-wave light from the foliage being approached, instead of turning away and thereby maintaining the previous 'steady-state' partition of the two kinds of light in its eyes. The term 'alighting flight' is strictly applicable only to the few seconds of approaching or hovering stimulated by a particular plant or similar object, for this is causally the opposite of maintained low-level plant-dodging flight. If Moericke's expressive term 'alighting flight' is to be retained, it were better reserved for those whole phases in the migrant's activity when it flies low, not in a constant state of readiness to alight, but passing intermittently into one. This can be contrasted with the phases of 'distance flight' when the phototactic response is so much stronger to the sky light than to the plant light that the insect soars.

Turning to the role of light reactions in natural alightments and hence in host selection, both the phototactic and the optomotor reactions could, in theory, serve flying aphids in distinguishing one kind of plant from another, by colour and by structure respectively. In practice it is clear that 'yellow-sensitive' species like *M. persicae*, *A. fabae* and *B. brassicae* do not distinguish their host plants by such means from a wide range of non-host plants. Aside from minor variations such as the slightly weaker yellow preference of *brassicae* (p. 10), the phototactic 'preference' for the yellowest foliage does not help them to distinguish host plants 'botanically', as members of a plant-taxonomic category. On the other hand, the measurements imply that it does serve them in distinguishing members of the broad plant-physiological category to which their host plants belong.

Prominent among 'yellow-sensitive' aphids are the heteroecious species alternating between dicotyledonous herbs in the summer and trees or shrubs in the autumn-winter-spring (Eastop, 1955; Heathcote, 1957; Lamb, 1958). According to the limited number of leaf reflectance measurements so far made, the aphids' colour preferences will help

them to alight on the right kind of plant, herb against tree or vice versa, at the right season, owing to the seasonal alternation in the relative yellowness of these two kinds of foliage. The phototactic colour preferences of some aphids may be more host-specific than that. After Moericke (1955*a*, *b*) had found in trap experiments that *Hyalopterus pruni* (Geoffr.) was peculiar among 'yellow-sensitive' species in preferring green or yellow containing some short-wave reflexion instead of pure yellow, he was disappointed to obtain a reflectance curve from the host leaf, *Phragmites*, that differed only very slightly in the expected direction from potato and beet leaves. But direct reflexion from the almost horizontally posed *Phragmites* leaves, together with the dead reed leaves and water that are common in natural reed beds, may, in nature, increase the difference in the expected direction.

On present evidence, however, the distinguishing of one kind of plant from another would seem to be a secondary, specialized function of colour vision in aphids. A glance at the leaf and sky curves on the foregoing pages brings out the fact that, whatever else it may do, sensitivity to yellow will serve to distinguish plants in general from the sky, no less than sensitivity to green. The key to this primary discrimination is simply the position of the dividing line at 500 m μ . And this discrimination, on the modified Moericke hypothesis, is the key to low-level 'alighting flight' and so to host finding. The 'yellow-insensitive' species which are not attracted to yellow traps and are referred to by Moericke (1957) as 'colour-insensitive', may nevertheless have the same type of colour vision (with the dividing line at 500 m μ .) serving the same general function. Sky light is so much richer in short-wave energy than plant light, that aphids with this type of colour vision could go much further than *H. pruni* in requiring short-wave energy admixed with long-wave to attract them to plants and still readily distinguish plants from sky.

It is therefore suggested that the primary function of colour vision in flying aphids lies in the distinguishing of plants from sky. Thanks to their changing colour responsiveness during flight, aphid migrants that have dispersed by surrendering themselves for a while to the upper air circulation, can regain contact with the earth's plant cover before they are exhausted, and can there sample the flora in a succession of alightments which as far as we know is their sole method of host finding. A striking analogy is the case of *Daphnia magna* which has the same type of colour vision and according to Smith and Baylor (1953) finds its green plankton food in the water in much the same way.

We are grateful for the help of Mr H. L. G. Stroyan in identifying aphids, of Mr R. P. Bumstead in field experiments, and of Messrs Unicam Instruments Ltd in spectrophotometry.

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Determination of the resistance of locusts to γ -BHC and diazinon in relation to their age, sex and weight

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(Received 31 March 1960)

SUMMARY

The relation between body weight and resistance to γ -BHC and diazinon was studied in experiments using the desert locust (*Schistocerca gregaria*) and the African migratory locust (*Locusta migratoria migratorioides*).

In all experiments with *Schistocerca*, resistance was proportional to weight within any one age and sex group. Resistance to diazinon (per unit body weight) decreased with age, that of the males at a faster rate than that of the females. Against γ -BHC the insects showed a nearly twofold increase in resistance about 1 week after fledging.

Within groups of *Locusta* the resistance to diazinon was found to be independent of weight, but the resistance to γ -BHC was dependent on weight. Similarly the resistance to diazinon at different ages after fledging was independent of age but resistance to γ -BHC increased with age.

INTRODUCTION

Previous work (MacCuaig, 1956*a*) has shown that the resistance of adults of the desert locust (*Schistocerca gregaria*) and of the African migratory locust (*Locusta migratoria migratorioides*) to DNC applied as a contact poison to the ventral surface of the abdomen is directly related to body weight irrespective of age or sex.

This paper describes similar experiments carried out to determine how the resistance of similar adult locusts to γ -BHC and diazinon is related to their age, sex and body weight.

MATERIALS AND METHODS

γ -BHC was applied as a 4 or 7% solution of the technically pure substance dissolved in kerosene extract bottoms (KEB), whilst diazinon was applied in a solution prepared by diluting the technically pure liquid (90% active ingredient) with KEB. The toxicity determinations were made in terms of the technically pure liquid and the solutions used contained 5 or 10% of this substance. The toxicity of solutions of both these insecticides has been found to depend only on the amount of active ingredient applied.

The insects used were the desert locust, *Schistocerca gregaria* (Forsk.), and the African migratory locust, *Locusta migratoria migratorioides* (R. & F.), derived from stocks bred in the laboratory of the Anti-Locust Research Centre, London. The rearing conditions were similar to those described by Hunter-Jones (1956). Significant differences between the resistance of different batches of locusts to these insecticides

are frequent and care was taken to distribute the insects of each batch used amongst the various treatment groups of each experiment.

The method of application, by means of the micro-drop syringe, has already been described (MacCuaig, 1956*a*). The locusts were used at known ages, age being measured from the time of fledging. Each insect was weighed before dosing and the insecticide applied as a single drop between the first and second abdominal sternites. In some experiments doses of the active constituent were applied regardless of the weight of the insects, whilst in others they were measured as weight of active constituent per

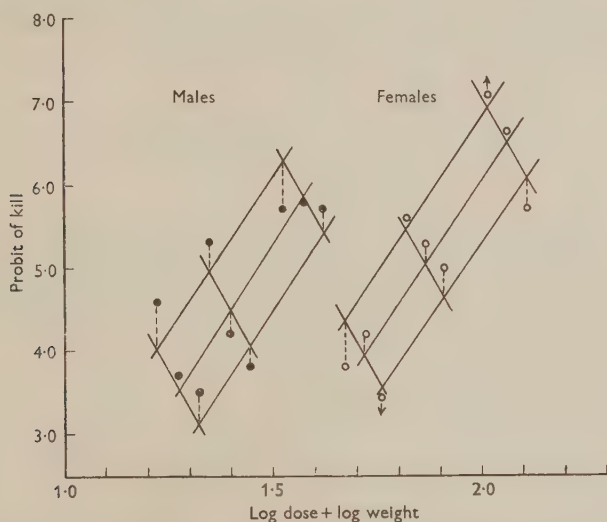


Fig. 1. Probit planes relating resistance of *Schistocerca* to diazinon in relation to weight.

gramme locust weight. After treatment, the locusts were kept under the relatively constant conditions of 24–27° C. and 60–80% relative humidity. The insects were recorded as 'potentially dead' when they could not right themselves after being turned upside down. Insects affected to this extent were never seen to recover.

The method of probit analysis has been used throughout (Finney, 1952), and the interaction of the two factors dose and weight was studied by means of a factorial analysis using the probit plane technique. In these experiments it was assumed that the joint effects of dose and weight could be represented by the equation

$$Y = b_1Q + b_2W + c, \quad (1)$$

giving the probit of kill (Y) in terms of the logarithm of the quantity of insecticide applied (Q) and the log of the weight. The experiments were designed to determine the value of the regression coefficients b_1 and b_2 . The locusts used for each experiment were divided into sections according to weight. Each section in turn was divided into

two or three groups each receiving different doses. Thus in each separate experiment the distribution of the insects was such that, after dosing, four, six, or nine groups were formed (according to the number of insects available), each differing from the others in either the quantity of insecticide applied or the mean weight (see Table 1). From the mortalities the best fitting probit plane was calculated. An example is shown in Fig. 1. The lines with positive slopes connect groups of equal weight and show the regression of probit of kill upon log dose, whilst the lines with negative slope show the regression of Y upon W when the dose is constant. For these experiments the sexes were treated separately, males and females usually receiving different doses, but the probit planes obtained for each were drawn parallel since there were never significant differences between corresponding regression coefficients.

RESISTANCE IN RELATION TO BODY WEIGHT

Experiments with diazinon

(a) *Schistocerca*

A single large batch of adults was used when they were between 19 and 26 days old. The insects were kept for 6 days after dosing, by which time the insecticide had completed its action. Controls were not used in this experiment but the natural mortality, if any, was clearly very low and was ignored. The experimental details, calculated regression coefficients and toxicities are given in Table 1, whilst the probit planes are shown in Fig. 1.

The residual heterogeneity χ^2_{10} was not large ($\chi^2 = 13.5: 0.5 > P > 0.25$), thus indicating that the probit plane represented by equation (1) adequately expressed the relationship between mortality probit, quantity of insecticide applied and the weight of the insects. The values of b_1 and b_2 are both significantly different from zero, indicating that both the quantity of insecticide applied and the weight of the insect exert a real effect on the kill obtained. The two coefficients are approximately equal numerically and their sum (-1.46) is not significantly different from zero, enabling equation (1) to be reduced to

$$y = bx + c, \quad (2)$$

where $b = b_1 = (-b_2)$ and $\log^{-1}x = \log^{-1}Q \log^{-1}W$. That is, the relationship between mortality probit and dosage is correctly expressed when the dose is measured in weight of insecticide per unit weight of locust. The LD 50's and LD 99's were obtained expressing the doses in $\mu\text{g. g.}$ and it was found that the females were more resistant than the males by a factor of 1.28, with standard error 0.07.

(b) *Locusta*

For these experiments a total of 438 insects were used in three experiments in which the males and females were treated separately. The complete data, together with the results of their analyses, are shown in Table 2. It will be seen that there is little decrease in percentage kill with increasing weight. The probit planes were all drawn parallel and common values of the slopes obtained. In contrast to the results obtained with *Schistocerca*, the regression of kill on weight was not significantly different from

zero. Thus in this case the resistance of the insects within each batch does not seem to be related to body weight, yet in spite of this when the average weights of the different groups of insects were compared with their resistances (LD₅₀'s in $\mu\text{g. locust}$) they were found to be very closely correlated (Fig. 2). Similarly, the resistance of different age groups (see later) is related to weight and is shown in the diagram.

Table 1. *Resistance and body weight; diazinon applied to Schistocerca gregaria*

Sex	Mean weight (g.)	Dose ($\mu\text{g.}$)	No. of insects	Mortalities	
				No.	Percentage
Males	2.229	7	14	5	36
		10	11	7	63
		15	16	12	75
	2.519	7	11	1	9
		10	18	4	22
		15	10	8	80
	2.801	7	14	1	7
		10	9	1	11
		15	12	8	75
	Total		115		
Females	3.177	15	18	2	11
		21	18	13	72
		33	18	18	100
	3.524	15	15	3	20
		21	15	9	60
		33	19	18	95
	3.927	15	17	0	0
		21	18	9	50
		33	15	12	75
	Total		153		

Regression coefficients and standard deviations:

b_1	b_2	$b_1 + b_2$
7.52 ± 0.80	-8.98 ± 2.44	-1.46 ± 2.41

Toxicity of diazinon ($\mu\text{g./g.}$):

	LD ₅₀	LD ₉₉
Males	4.55	8.73
Females	5.83	11.2

(a) *Schistocerca*

Experiments with γ -BHC

The data for this analysis were obtained in a different manner from the previous ones. When sufficient results—that is, individual mortality records—had accumulated from ordinary toxicity experiments using γ -BHC, they were divided into three sections according to weight and each in turn was divided into three groups according to the dose applied. Formation of the groups was not entirely random since some selection was made to bring the three sets of doses to about the same means but no selection was

Table 2. *Resistance and body weight; diazinon applied to Locusta migratoria*

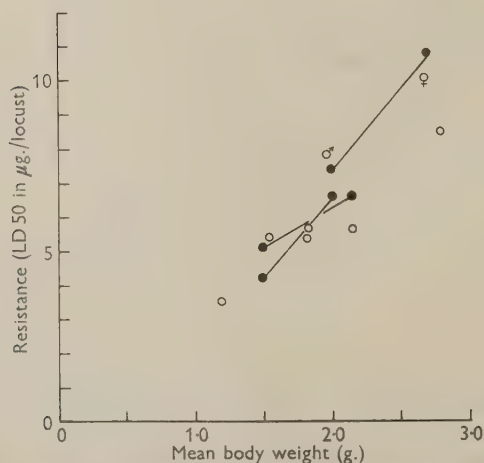
Experiment no.	<i>n</i> = number of insects in group											
	1				2				3			
	Light		Heavy		Light		Heavy		Light		Heavy	
Mean weight (g.)	1.36		1.64		1.35		1.66		1.85		2.13	
Dose applied ($\mu\text{g./locust}$)	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
3.0	13	23	13	46	11	0	17	12	9	11	9	22
5.5	13	69	12	58	14	57	13	46	8	25	9	11
9.5	12	92	12	92	12	100	16	94	8	50	9	78
Females												
Mean weight (g.)	1.78		2.22		1.93		2.31		2.54		2.90	
Dose applied ($\mu\text{g./locust}$)	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
4.5	13	23	13	7	13	0	15	27	11	0	12	8
7.5	12	58	12	54	17	65	10	30	11	27	12	8
12.5	13	92	12	84	17	100	10	80	11	33	12	67

Regression coefficients and standard deviations:

$$\begin{array}{ccc}
 b_1 & b_2 & b_1 + b_2 \\
 4.32 \pm 0.39 & -1.04 \pm 1.74 & 3.28 \pm 1.79
 \end{array}$$

Toxicity of diazinon (LD 50 $\mu\text{g./locust}$):

Experiment	1	2	3
Males	4.17	5.06	7.41
Females	6.59	6.59	10.8

Fig. 2. Relation between resistance to diazinon and body weight of different batches and sexes of *Locusta*. Experiments: ●, resistance and body weight; ○, resistance and age.

made on the basis of kill, so that the validity of the results was unaffected. Experiments with γ -BHC generally last 10 days and the natural mortality of the insects over this period was estimated at 10% for each sex from an examination of all the original data from which the results were drawn. Thereafter the procedure exactly followed that of the previous experiment with *Schistocerca*. The data obtained, together with the results of their analyses, are shown in Table 3.

Table 3. *Resistance and body weight; γ -BHC applied to Schistocerca gregaria*

Percentage mortality corrected for a control mortality of 10 %

Sex	Mean weight (g.)	Dose (μ g.)	No. of insects	Mortalities	
				No.	%
Males	1.72	8.0	32	9	20
		11.7	21	13	58
		17.2	28	23	80
	2.03	8.0	21	4	10
		11.7	23	6	18
		17.2	41	27	62
	2.35	8.0	25	3	2
		11.7	47	19	33
		17.2	33	15	39
	Total		271		
Females	2.64	12.3	25	8	24
		19.8	38	20	48
		28.0	14	12	84
	3.02	12.3	20	4	11
		19.8	37	20	49
		28.0	21	14	62
	3.61	12.3	18	3	8
		19.8	25	10	33
		28.0	23	15	61
	Total		221		

Regression coefficients and standard deviations:

b_1	b_2	$b_1 + b_2$
4.32 ± 0.63	-4.87 ± 1.36	-0.55 ± 1.20

Toxicity of γ -BHC (μ g./g.):

	LD 50	LD 99
Males	7.2	23
Females	7.1	21

As in the previous experiments, the heterogeneity introduced by constraining the planes for males and females to lie parallel with one another was negligible. The residual heterogeneity χ^2 was small and the regression coefficients were clearly significantly different from zero. Again the equation for the probit plane may be reduced to the form of equation (2). To γ -BHC, however, the sexes are equally resistant when the doses are measured in μ g./g.

(b) Locusta

As with the experiments in which *Schistocerca* were dosed with diazinon a single large batch of insects was used. The regression of kill on weight was again highly significant as is shown in Table 4.

Table 4. *Resistance and body weight; γ -BHC applied to Locusta migratoria*

Regression coefficients and standard deviations:

b_1	b_2	$b_1 + b_2$
4.17 ± 0.61	-8.34 ± 2.03	-4.17 ± 2.00

Toxicity ($\mu\text{g./g. locust}$):

	LD ₅₀	LD ₉₉
Males	5.2	19
Females	4.1	14.7

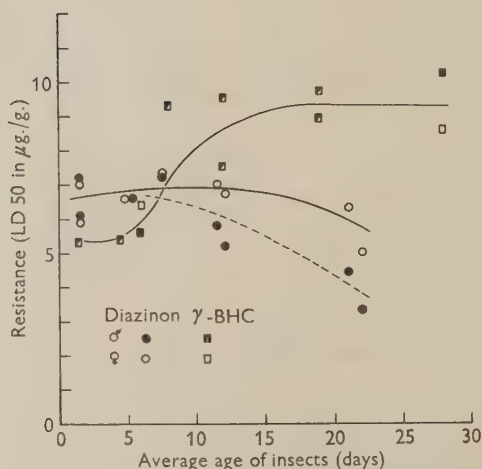


Fig. 3. Resistance of *Schistocerca* to γ -BHC and diazinon in relation to age and sex.

This analysis suggests that a decreasing weight has a significantly greater effect on kill than a proportionately increasing dose. In fact, because the slope of b_2 is numerically exactly twice that of b_1 the toxicity in terms of $\mu\text{g./unit body weight}$ is directly proportional to body weight, that is, the LD₅₀ for a 3 g. locust is nine times that of one weighing only 1 g.

RESISTANCE IN RELATION TO AGE

For the experiments using diazinon against *Schistocerca* 849 insects were dosed in three experiments at different ages as shown in Table 5. The LD₅₀'s obtained are also shown in Fig. 3 where they are plotted against age. Up to 1 week after fledging it will

be seen that there is no difference between the sexes in their resistance to doses measured in $\mu\text{g./g.}$ After that time the resistance of the males decreases to about half its original value and that of the females to about 80% of its original value. The experiments were carried out in such a manner that they could be analysed factorially as in the previous sections by fitting a series of probit planes, and it was again found that

Table 5. *Relation between adult age and resistance of Schistocerca gregaria to diazinon*

Age (days)	Sex	No. of insects	Mean weights (g.)	LD 50 ($\mu\text{g./g.}$)	LD 99 ($\mu\text{g./g.}$)
1-2	♂	60	1.42	7.2	16.3
	♀	60	1.82	5.9	13.2
	♂	31	1.53	6.1	11.9
	♀	39	1.86	7.0	13.7
3-7	♂	73	1.93	6.6	14.7
	♀	65	2.45	6.6	14.8
7-8	♂	48	1.75	7.2	16.1
	♀	48	2.47	7.3	16.4
10-13	♂	51	2.37	5.8	12.9
	♀	53	2.98	7.0	15.7
11-13	♂	44	2.30	5.2	11.6
	♀	44	3.24	6.7	15.0
19-23	♂	51	2.20	4.4	9.7
	♀	50	3.09	6.3	14.0
21-23	♂	74	2.32	3.3	7.4
	♀	58	3.25	5.0	11.3

Table 6. *Relation between adult age and resistance of Schistocerca gregaria to γ -BHC*

Age (days)	Sex	No. of insects used	Mean weights (g.)		LD 50 ($\mu\text{g./g.}$)	LD 99 ($\mu\text{g./g.}$)
			Males	Females		
0-3	Mixed	45	1.57	1.87	5.25	11.7
3-6	Mixed	43	1.66	2.04	5.38	12.0
2-10	Males	40	1.66	—	5.64	12.6
2-10	Females	40	—	2.20	6.37	14.2
7-9	Mixed	48	2.08	2.68	9.33	20.8
11-13	Mixed	48	2.40	3.11	9.55	21.3
10-14	Mixed	52	2.60	3.76	7.50	16.7
18-20	Mixed	48	2.16	3.01	9.73	21.7
17-21	Mixed	48	2.46	3.47	8.93	19.9
24-32	Males	40	2.39	—	10.21	22.8
24-32	Females	40	—	3.92	8.59	19.2

for each sex at each age group the regression of kill on weight was significantly different from zero, but the sum of the two regression coefficients was never significantly different from zero (MacCuag, 1956b).

With γ -BHC, four batches of insects were used, each distributed over at least two age groups. The details are shown in Table 6 and in Fig. 3. The probit regression lines

were all drawn parallel since significant heterogeneity was not introduced by doing this ($\chi^2_{(1)} = 10.5$). For the four youngest groups the LD 50's ranged from 5.25 to 6.37 $\mu\text{g./g.}$ From 8 days onwards the LD 50 had increased to a mean value of 9.12 $\mu\text{g./g.}$ and did not overlap the LD 50's from the younger insects. It thus appears that after the first 8 days or so the resistance of the insects increases fairly sharply to a value which remains constant during the next 3 weeks. At 2-10 and 24-32 days the number of insects used was sufficient to enable the sexes to be treated separately. Only small differences occurred between them at either age.

Using diazinon against *Locusta* at the ages of 1-2, 7-9, 11-14 and 26-30 days the LD 50's were remarkably constant, ranging only from 2.6 to 3.5 $\mu\text{g./g.}$ When the LD 50's in $\mu\text{g./locust}$ were plotted against the mean weight of the group they were found to fit the results obtained in the previous section, using *Locusta* with diazinon, and are therefore added to Fig. 2 without separation into age groups. However, in experiments with γ -BHC against the same species there were large differences in the resistance of young and mature insects. The younger adults were on each occasion more than twice as susceptible to doses measured in $\mu\text{g./g.}$ (Table 7).

Table 7. *Relation between adult age and resistance of Locusta migratoria to γ -BHC*

Age (days)	No. of insects	Mean weight (g.)		LD 50 ($\mu\text{g./g.}$)	LD 99 ($\mu\text{g./g.}$)
		♂	♀		
3-5	66	1.09	1.49	3.83	14.5
	54	1.35	1.68	3.52	10.7
16-19	34	1.73	2.21	8.8	33
	60	1.64	2.22	9.0	27

DISCUSSION

These experiments can best be considered in relation to the results of similar ones using DNC, in which the resistance of both *Schistocerca* and *Locusta* was found to be proportional to weight independently of both age and sex. Thus the resistance of *Schistocerca* has now been shown to be directly related to weight for three different types of insecticide, a nitrophenol, an organo-phosphorus compound and a chlorinated hydrocarbon.

In one case (γ -BHC), resistance was found to be dependent on age as well as weight and in another (diazinon) on age, weight and sex.

The results with *Locusta* are rather more variable. Resistance within groups is not related to weight with diazinon, is related only to weight with DNC, and is related to weight and age with γ -BHC. In view of this variation it is not surprising that both Hopf (1951), and Stringer (1956), using *Locusta* dosed with a variety of insecticides, found that the best fitting dosage/mortality curves were obtained by applying doses of insecticide per locust regardless of individual variations in the weight of the insects used.

The toxicity of γ -BHC and diazinon to fifth-instar nymphs (hoppers) of *Schistocerca*

has been determined at various ages after moulting (MacCuaig, 1960). The resistance of the hoppers to γ -BHC was at a maximum (11.0 $\mu\text{g./g.}$ in the middle of the instar), 2-5 days after moulting, falling to an average value of 5.7 $\mu\text{g./g.}$ 6 days later. This compares with the values found in these experiments of 5.2-6.4 $\mu\text{g./g.}$ for the adults just after fledging which then increased to 9.1 $\mu\text{g./g.}$ shortly before maturity. It is thus apparent that resistance to γ -BHC decreases a few days before and after moulting.

To diazinon, fifth-instar hoppers are generally much more resistant than adults; the LD 50's up to 7 days after moulting averaged 12.6 $\mu\text{g./g.}$ but at 8-9 days were only 6.6-8.5 $\mu\text{g./g.}$ These last values are similar to the resistance of adults 1-2 days after fledging where the LD 50 ranged from 5.9 to 7.2 $\mu\text{g./g.}$ Thus with diazinon the decreased resistance occurring before the moult remains, until on maturity the insects become even less resistant, the average LD 50 for mature *Schistocerca* of mixed sexes being 4.7 $\mu\text{g./g.}$

Thanks are due to Mr W. S. Watts and Miss M. Yeates who carried out most of the experimental work described, and to Dr T. H. C. Taylor for his help in preparing the paper for publication.

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Effect of time and temperature on toxicity of insecticides to insects

I. Tests of DDT on larvae of *Aedes aegypti* L.

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(Received 5 March 1960)

SUMMARY

The effects of a change in temperature (15-28° C.) on the toxicity of dilute suspensions of DDT to larvae of *Aedes aegypti* L., as assessed by a photomigration method, depended on the stage of the test during which the temperature was changed. Increase in temperature during exposure to DDT (0.02 p.p.m. for about 1 hr.) increased the toxic action. When larvae were left in the suspensions for the duration of the test (3 hr.-4 days), increase in temperature throughout the test decreased the toxic action of a very low concentration of DDT (0.002 p.p.m.), but had no effect with higher concentrations (0.1-0.2 p.p.m.). Toxic action was greater in larvae held at a low temperature than in larvae held at a high temperature after treatment (0.025 p.p.m. for 3 hr.). Such toxic action was reversible: a change from high to low temperature increased paralysis, and larvae, paralysed at a low temperature, recovered when the temperature was raised.

INTRODUCTION

A change in temperature may increase or decrease the toxic action of an insecticide to insects, and a poison is said to have a positive or negative temperature coefficient according to whether its toxic action is increased or decreased by a rise in temperature. Häfliger (1954) and Pradhan & Mundkur (1957) have distinguished between 'pre-treatment', 'treatment', 'post-treatment' and 'continuous treatment' temperatures; in any one test there can be several different temperature coefficients, which may be more or less distinct, according to how clearly the design of the test separates the different phases.

To these special terms some others can be added; they apply in particular to DDT, which usually has a negative post-treatment temperature coefficient of toxicity and often has a negative continuous treatment coefficient. However, these coefficients depend on the dose rate (e.g. applied concentration); they may not be found with high rates and may even be replaced by positive coefficients. This 'dose effect' was first noticed by Fan, Cheng & Glenn Richards (1948) in tests by continuous treatment,

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and was found, for various coefficients of DDT, by Hoffman & Lindquist (1949), Woodruff (1950), Barker (1957) and by Kuenen, den Boer & de Melker (1957).

Insects, paralysed at a low post-treatment temperature by a suitable dose of DDT, can recover when put at a higher temperature, and become paralysed again when re-cooled. The whole cycle can be repeated several times. This well-known effect, first noticed by Lindquist, Wilson, Schroeder & Madden (1945), is referred to here and in later papers as 'reversibility of action'. It is distinct from 'inversion of temperature coefficient', or the change in post-treatment or continuous treatment coefficient from negative to positive at extreme temperatures (Kaeser, 1948; Barker, 1957; Pradhan & Rangarao, 1957).

This paper is about the effects of temperatures on the toxicity of DDT to larvae of *Aedes aegypti* L., with assessment of results by a photomigration method. The effects of treatment, continuous treatment, and post-treatment temperatures are distinguished; the results show both the dose effect and reversibility of action. (Some tests on inversion of temperature coefficient of DDT on another species are given in a later paper.)

MATERIALS AND GENERAL METHODS

A. aegypti, reared by the method of Burchfield, Redder, Storrs & Hilchey (1953), were used as third- or fourth-instar larvae. Dilute suspensions of DDT (m.p. 106–107° C.) were made by pipetting a 0.01 % stock solution in 95 % alcohol into well-stirred distilled water or nutrient solution (0.025 % total solids as brewer's yeast: blood albumen:sucrose = 5:3:2). The highest concentration of alcohol was 0.2 %.

DDT was applied to the larvae by immersing them in the suspensions. After treatment, toxic action was assessed by the photomigration method of Burchfield, Hilchey & Storrs (1952), with $9 \times 1\frac{3}{4}$ in. glass troughs, at the same temperatures as those of continuous treatment or post-treatment. Larvae which could not travel more than 3 in. in 1 min. were counted as paralysed; normal larvae could travel at least 9 in. in less than 1 min., even at 15° C.

Other details are given below.

DETAILS AND RESULTS

Treatment temperature

Suspensions were made in distilled water at 0.02 p.p.m. at 15° C. and 25° C., and larvae were treated in specimen tubes containing 50 ml. suspension. Fifteen third-instar larvae were placed in each tube for treatment times of 2.5–256 min., with two or four replicate tubes for each treatment time at each temperature. At the end of the treatment time, the larvae were sieved from the suspensions, washed in distilled water and kept in nutrient solution at 20° C. for 3 days. Toxic action was assessed daily at 20° C. The proportion paralysed in the controls did not exceed 6 %.

The probits of the corrected percentages paralysed (Abbott, 1925) were plotted against logarithms of treatment times; there were four or five points for each treatment temperature. The treatment times giving 50 % paralysed (ET₅₀) were read from straight lines fitted to the points by eye. Each ET₅₀ was based on about 230 larvae.

Table 1 shows the results from the 2-day counts of four tests; the results from the 1- and 3-day counts were only very slightly different. The 95% confidence limits (Litchfield & Wilcoxon, 1949) are shown in roman type on either side of each ET 50 in minutes. The treatment temperature coefficients were clearly positive; the geometric mean of the ratios of ET 50's at the two temperatures was 3.9.

Continuous treatment temperature; dose effect

Suspensions were made in nutrient solution at 0.2, 0.1 and 0.002 p.p.m. at 15 and 28° C. Four replicates of ten fourth-instar larvae were used for each concentration at each temperature. Toxic action was assessed at intervals for up to 3 hr. (0.2 and 0.1 p.p.m.) or 4 days (0.002 p.p.m.) at the treatment temperatures.

Table 1. *Effect of treatment temperature on time to cause 50% paralysis of 3rd-instar Aedes aegypti larvae, kept at 20° C. for 2 days after treatment with 0.02 p.p.m. DDT suspensions*

Test	Treatment temperature (° C.)	ET 50 (min.), with 95 % confidence limits		
1	25	16	19	22
	15	24	45	85
2	25	8	10	13
	15	24	28	33
3	25	6	10	18
	15	57	70	85
4	25	9	11	13
	15	35	53	80

In the tests at 0.2 and 0.1 p.p.m., the suspensions and larvae were kept for the first hour in the glass troughs of the Burchfield apparatus. After this, they were moved to corked specimen tubes between counts. In the test at 0.002 p.p.m., specimen tubes were used between counts throughout the test, and the suspensions were renewed daily.

Fig. 1 shows the results. The figures for the test at 0.002 p.p.m. were corrected for the percentages paralysed in the controls, which did not exceed 20% on the 4th day. The figures for the other tests were not corrected; in our experience, the number of larvae paralysed in 3 hr. at either 15° C. or 28° C. very seldom exceeds 2%. The continuous treatment temperature coefficient was large and negative at 0.002 p.p.m., but there was a clear dose effect: at 0.1 and 0.2 p.p.m., temperature had no effect on toxicity.

Post-treatment temperature; reversibility

Batches of fifteen larvae were exposed to suspensions (0.025 p.p.m.) in distilled water in 50 ml. specimen tubes for 3 hr. at 28° C. After this the larvae were washed in distilled water and kept in fresh nutrient solution. In each of two tests (5 and 6), four randomly chosen batches were put at 28° C., and four at 15° C., for another 6 hr., after which toxic action was assessed at 28° C. The larvae were then changed to more fresh nutrient solution, and those which had been at 28° C. were moved to 15° C., and vice

versa, for the final 14-16 hr. of the test; and toxic action was again assessed at 28° C. In each of two other tests (7 and 8), four batches were put through the 28-28-15° C. sequence only. In each test, there was an untreated control batch for every treated batch.

Table 2 shows the results. Each figure is based on four batches of fifteen larvae, and is corrected for the percentages paralysed in the controls ($5 \pm 4\%$); the standard

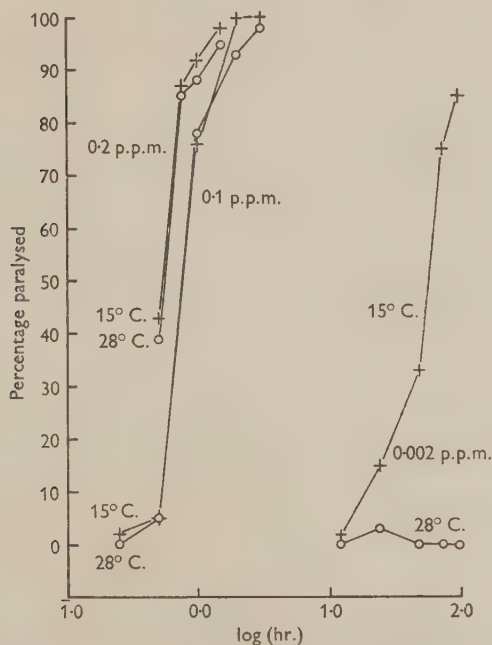


Fig. 1. Effect of concentration of DDT suspensions, continuous treatment temperature, and time on the percentage paralysis of *A. aegypti* larvae.

Table 2. Effect of changes in post-treatment temperature on percentage paralysis of 3rd-instar *Aedes aegypti* larvae after exposure to 0.025 p.p.m. DDT suspensions for 3 hr. at 28° C.

Test	Percentage paralysed <i>n</i> hr. after end of treatment, at temperatures shown	
	6 hr.	20-22 hr.
5	59 ± 14	28° C. → 15° C. { 69 ± 7 77 ± 13 65 ± 8 87 ± 11
6	65 ± 13	
7	40 ± 4	
8	74 ± 12	
5	97 ± 4	15° C. → 28° C. { 76 ± 12 80 ± 9
6	99 ± 3	

errors are approximate. Comparison of the figures by Wilcoxon's (1949) ranking method for groups of replicates showed that the two sets of figures from the first assessments in tests 5 and 6 differ significantly; there was a clear negative post-treatment temperature coefficient. Similarly, the percentage of larvae paralysed increased significantly between the first and second assessments when the post-treatment temperature was decreased from 28 to 15° C. (all tests), and vice versa (tests 5 and 6); the DDT-treated larvae showed reversibility of action.

DISCUSSION

The results found here are in line with those of other authors.

The treatment temperature coefficient is usually positive in tests with flying or crawling terrestrial insects exposed to DDT films (Lindquist *et al.* 1945, 1946; Pradhan, 1949; Loschiavo, 1955; Pradhan & Srivastava, 1956; Hadaway, 1957; Kuenen *et al.* 1957). Our results with aquatic larvae conform with the general finding, no doubt because 'pick-up' of DDT is increased by raising the temperature (Pradhan & Srivastava, 1956). This pick-up factor also has its effect in tests by continuous treatment, in which the treatment and post-treatment temperatures are not separated.

The dose effect in the tests by continuous treatment is less than that obtained by Fan *et al.* (1948), who also used *A. aegypti*; this is possibly because they used a different method to assess toxic action, or because the strains differed.

In our tests, temperature had no effect on toxicity at 0.2 and 0.1 p.p.m.; Fan *et al.* found a clear positive coefficient at 0.1 p.p.m. Had we used concentrations greater than 0.2 p.p.m., we might have found a positive coefficient, just as Pradhan & Mundkur (1957) might have found a dose effect in their tests on larvae of *Culex fatigans* had they used even lower concentrations.

Pradhan (1950) suggested an explanation for the dose effect found by Fan *et al.* He supposed that penetration by DDT into insects has a positive temperature coefficient, that the rate of penetration increases with concentration of poison in suspension, and that the temperature coefficient of resistance is positive. The observed temperature coefficient of toxicity depends on the balance between rate of penetration and resistance. In any temperature range, the observed temperature coefficient may therefore be positive when the applied concentration of DDT is high, or negative when it is low.

Some of Pradhan's assumptions have been confirmed by other workers. Tests where measured doses were applied to insects showed the post-treatment temperature coefficient of penetration of insect cuticle by DDT to be positive. The post-treatment temperature coefficient of metabolism of DDT inside insects is negligible or positive, but never large enough to offset the positive coefficient of penetration. The result is that an increase in post-treatment temperature nearly always increases the net amount of unchanged DDT inside the insects. In spite of this, the observed post-treatment temperature coefficient of toxicity is nearly always negative (Vinson & Kearns, 1952; Tahori & Hoskins, 1953; Wiesmann, 1955; Barker, 1957; Menn, Benjamini & Hoskins, 1957).

These facts come from tests in which measured doses were applied to the insects.

However, in tests by continuous treatment, which involve the pick-up factor, the temperature coefficient of penetration is likely to be even larger. So, according to Pradhan's ideas, tests by continuous treatment are particularly likely to show a dose effect. Similarly, Menn *et al.* (1957), considering the results of their exact work on metabolism of DDT, concluded that its 'toxicity will have a negative temperature coefficient if the dose is moderate in size and given but once'.

We wish to acknowledge the guidance given by Dr A. H. McIntosh throughout this work. We are also indebted to Dr C. Potter for his suggestions and constructive criticism.

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Effect of time and temperature on toxicity of insecticides to insects

II. Tests of DDT on adult *Tenebrio molitor* L. below 10° C.

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(Received 20 March 1960)

SUMMARY

The toxicity of DDT to insects usually increases as the post-treatment temperature is decreased in the commonly used temperature range (30-10° C.). The toxicity increased further when the post-treatment temperature of adult *Tenebrio molitor* L., dosed by topical application, was decreased from 10 to 6° C. However, when the post-treatment temperature was decreased still more (down to -1° C.), the toxicity by topical application or by injection apparently decreased again. The size of this 'positive temperature coefficient' below 6° C. decreased as time passed after treatment. Differences in toxicity below 6° C. were more apparent than real, and were the result of differences in the speeds at which symptoms appeared.

The slight toxic action at very low temperatures was evidently not caused by failure of DDT to penetrate the cuticle, or to reach its site of action. More probably, the speed of the chemical or physical process which actually caused paralysis was extremely low at the very low temperatures.

INTRODUCTION

The effects of temperature on the toxicity of DDT to insects have not usually been measured below about 10° C. Many insect species, although almost inactive below about 10° C., can survive exposure to much lower temperatures; adult mealworms (*Tenebrio molitor* L.) can survive several days at -1° C. The effect of the usual range of 'post-treatment' temperatures (Pradhan & Mundkur, 1957) on the toxicity of DDT to these insects conforms with what is now a well-known pattern: the toxicity increases as the post-treatment temperature is decreased from 28 to 10° C. (Das & McIntosh, 1961).

This paper is about the effects of lower post-treatment temperatures (down to -1° C.) on the toxicity of DDT to adult *T. molitor*.

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MATERIALS AND GENERAL METHODS

All tests (except those in which the post-treatment temperatures were changed from 28° C.) followed the general design of probit assays (Finney, 1952).

T. molitor were reared on bran and potatoes at 20° C. Droplets of solutions of DDT (m.p. 106–107° C.) in highly purified ('Analar') acetone were topically applied to or injected into 2-week-old adults, at room temperature, with a micrometer syringe. Each insect was given, without anaesthesia, 3 μ l. of solution through a cannula pushed under its elytra (topical application), or 1 μ l. through a sharp 26-gauge needle inserted through the dorsal cuticle on the right side of the abdomen (injection).

After being dosed, batches of fifteen insects were put in 9 cm. Petri dish pairs each containing a circle of filter-paper; the dishes were at once moved to the post-treatment temperatures. Some sliced potato was put in the dishes beside the insects when they were to be kept for more than 4 days.

The insects were counted, at room temperature, after a few days; in some tests, the same insects were counted several times, at intervals of a few days. As it was nearly impossible to distinguish paralysed from normal insects at 10° C. or below, the insects at these temperatures were moved to room temperature for a short while before being counted, and were at once put back at the same post-treatment temperatures after counting.

There were at least two batches of fifteen insects for each concentration of DDT at each post-treatment temperature in each test. The percentages paralysed were calculated by the method of Tattersfield, Gimingham & Morris (1925), and were corrected for control deaths by the method of Abbott (1925). Probit lines were fitted by eye except where noted, and values for ED 50, the doses causing 50% paralysis, were read from these lines.

DETAILS AND RESULTS

Simple preliminary tests were done by topical application and injection. In each series, four batches of fifteen insects were used for each of four or five concentrations at each post-treatment temperature (–1 and 10° C.). After 4 days (topical application) or 3 days (injection), the insects were moved to room temperature for 1½ hr. and counted. Control insects were unaffected.

The ED 50's from the insects kept at 10° C. were 0.49 μ g. per insect (topical application) and 0.42 μ g. per insect (injection). The highest doses used on the insects kept at –1° C., with the resulting percentages paralysed, were 15 μ g., 17% (topical application), and 20 μ g., 30% (injection).

These tests were not followed to their end-points (Beard, 1949), but it is clear that the toxic action of DDT after 3–4 days did not increase indefinitely as the post-treatment temperature was decreased below 10° C. The temperature coefficient between –1 and 10° C. was positive by topical application and by injection, although it is nearly always negative in the temperature range above 10° C. This is an example of 'inversion of temperature coefficient' of DDT (Das & Needham, 1961). The following tests show something of its nature.

Temperature of maximum toxicity; effect of time

In each of three identical tests by topical application, two batches of fifteen insects were used for each concentration at each post-treatment temperature (2, 6 and 10° C.). The insects were kept at their post-treatment temperatures for 12 days, moved to 28° C. for 2½ hr., and counted. This was repeated on the 16th and 20th days. The highest percentage of insects killed in the controls was 22% after 20 days at 2° C.

The results of the three tests were very similar. The figures for the percentages paralysed were combined, and nine probit lines were drawn, one for each of three post-treatment temperatures at each of three counting times. Fig. 1 shows the probit

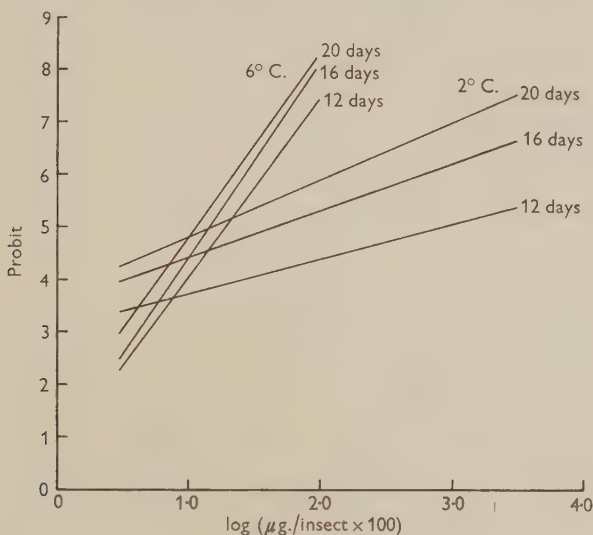


Fig. 1. Results of tests by topical application of DDT solutions to adult *T. molitor* at two post-treatment temperatures. The figures at the ends of the probit lines are the time intervals between treatment and counting.

lines obtained from the insects at 2 and 6° C. For simplicity, the lines from the insects at 10° C. are not shown; they were parallel to and very slightly to the right of the corresponding 6° C. lines. Six points were used for each 6 and 10° C. line, and eleven for each 2° C. line. None of the points is shown, however, but the lines are not extrapolated beyond the lowest and highest doses used. The 6° C. (and 10° C.) lines were fitted by eye; the positions of the 2° C. lines were calculated by the method of Finney (1952).

DDT was more toxic at 6° C. than at 10° C., after 12, 16 and 20 days, by a constant factor of about 1.5 at all dose levels; the temperature coefficient in this range, although small, was negative, as it is in the range above 10° C. Comparisons between 2 and 6° C.

are more complicated, because the two sets of lines are not parallel. The temperature coefficient between 2 and 6° C. was positive at nearly all dose levels in the 12-day counts; the ratio of ED 50's was 45. The decrease in ED 50 and increase in slope after the 12th day were larger at 2° C. than at 6° C. but, even on the 20th day, the 2 and 6° C. lines had still not become parallel; as a result, the temperature coefficient between 2 and 6° C. on the 20th day was negative at doses which gave less than 43% paralysis, and positive at higher doses. This is an example of the 'dose effect' (Das & Needham, 1961).

Changes of post-treatment temperature from 28° C.

In each of two identical tests by topical application, all insects received the same dose of DDT (1.5 µg.). The insects were afterwards kept, in nine batches of fifteen, for 12 hr. at 28° C.; none became paralysed in this time. Three batches were then

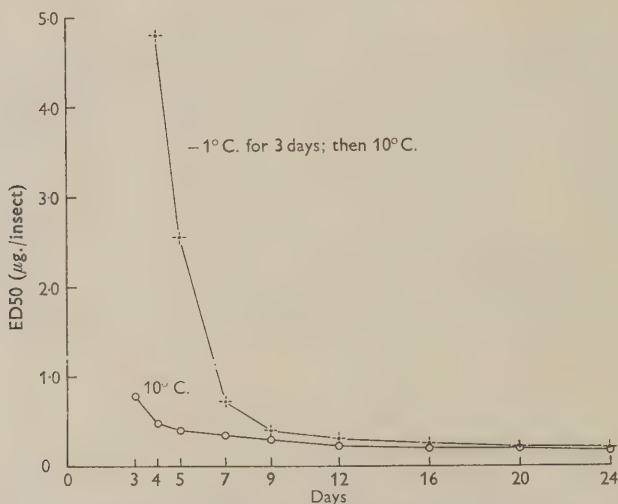


Fig. 2. Results of tests by topical application of DDT solutions to adult *T. molitor* at different post-treatment temperatures.

put at -1° C., three at 10° C. and three at 28° C. for 4 more days, moved to room temperature for 1½ hr., and counted. Two batches of fifteen served as controls at each final temperature; very few (> 7%) of these insects died.

The results of the two tests were very similar; the combined corrected percentages paralysed were 0% at 28° C., 92% at 10° C. and 0% at -1° C. The insects could tolerate 1.5 µg. at 28° C., were paralysed when cooled to 10° C., but remained unaffected when cooled directly to -1° C. The insects cooled to 10° C. showed 'reversibility of action' (Das & Needham, 1961).

Change of post-treatment temperature from -1°C .

The insects were kept without food at -1 and 10°C . for 24 hr. before treatment; they were dosed at room temperature by topical application, and put back at once at -1 or 10°C . In each of two identical tests, two batches of fifteen insects were used for each concentration at each post-treatment temperature; six or seven concentrations were used for each -1°C . probit line, and four for each 10°C . line.

The treated insects were kept, without food, at -1 and 10°C . for 3 days, moved to room temperature for $1\frac{1}{2}$ hr., and counted. Those which had been at -1°C . were then moved to 10°C ., with food, for the rest of the test; those which had been at 10°C . were given food and left at 10°C . All insects were then counted at intervals until the 24th day after treatment. The highest percentage of insects killed in the controls was 7% after 24 days.

Fig. 2 shows the geometric means of the ED 50's from the two tests, plotted against time after treatment. After 3 days at -1°C ., the insects showed no symptoms of DDT paralysis, but became paralysed after transfer to 10°C . They behaved, in fact, as if they had been dosed on the third day and placed directly at 10°C . The two curves in Fig 2 are, so far as can be judged, identical in shape and differ only in their positions on the time scale; the two final ED 50's were almost exactly the same.

DISCUSSION

Inversion of the post-treatment temperature coefficient from negative to positive at very low temperatures, found in these experiments, was also found by Barker (1957), with DDT-resistant house flies. At the other end of the temperature range (over 30°C .), Kaeser (1948), Barker (1957) and Pradhan & Rangarao (1957) found similar inversions of the post-treatment temperature coefficients with honey bees, DDT-resistant house flies and flour beetles.

However, extreme temperatures have in fact seldom been used in temperature-coefficient tests of DDT, and inversions at either end of the temperature range, whatever their cause, may turn out to be common rather than exceptional with insects which can withstand the necessary extreme temperatures. Adults of our strains of *Tribolium castaneum* Hb., *Oryzaephilus surinamensis* L. and a normally susceptible strain of *Musca domestica* L. could not survive more than a few days at -1°C . and so were not suitable for tests at low temperatures (cf. Solomon & Adamson, 1955).

Cause of decreased toxicity at very low temperatures

After a dose of DDT has been applied to the cuticle of an insect, some of it must (a) penetrate the cuticle, (b) be transported to its site of action and (c) take part in some chemical or physical process which causes paralysis.

The evidence discussed below shows that the most probable reason for the poor toxic action below about 6°C . is that stage (c) proceeds very slowly indeed, although it may eventually be as complete as at high temperatures. Thus, when insects are counted long before the end-points are reached (Beard, 1949), the observed differences

in toxicity (e.g. between 2 and 10° C.) may in fact be measures of differences in the speeds at which symptoms appear, rather than real differences in toxicity.

Supporting evidence. Wiesmann (1955) found that the penetration of DDT into DDT-resistant house flies was surprisingly large in 24 hr. at 5° C. Similarly, my results from the test by injection show that the slow reaction of the insects at -1 and 2° C., when dosed topically, was not necessarily caused by failure of DDT to penetrate the cuticle (stage (a)).

Fig. 2 shows that the final ED 50's from the two sets of insects were almost exactly the same. So the low toxicity below 6° C. is certainly not due to increased metabolism of DDT, which can evidently penetrate the cuticle at these temperatures.

In the tests where the post-treatment temperatures were changed from 28° C., the insects became paralysed within 4 days when moved to 10° C., but not when moved to -1° C., or when left at 28° C. Unaltered poison was evidently present at the site of action in the insects at 10° C. (stage (b)), and presumably also in those at -1° C., although it caused no symptoms in 4 days at this temperature.

At any one post-treatment temperature, the ED 50's of many contact poisons decrease with the passage of time after dosing, finally reaching steady values at the end-points (Beard, 1949). A later paper (Das & McIntosh, 1961) will show that a change in post-treatment temperature may affect the time taken for the end-point to be reached, or the size of the ED 50 at the end-point, or both. In contact tests on *T. molitor* in particular, the end-point was reached on the 5th day after treatment at 28° C., but not before the 24th day at 10° C., and the final ED 50 was much smaller at 10° C. than at 28° C. The post-treatment temperature coefficient was negative and increased in size with time after treatment.

Fig. 1 shows that when the post-treatment temperature was below 10° C., the same general pattern was followed. The end-points were not reached until at least the 20th day but, to judge from the rates of change of ED 50, the end-point was more closely approached at 6° C. (or 10° C.) than at 2° C. Had it been possible to keep the control insects alive even longer, the ultimate toxicity might have become greater, at all dose levels, at 2° C. than at either of the other two temperatures. The positive coefficient in the 12-day counts, and at high doses in the 16- and 20-day counts, would then be seen to be an expression of large differences in the speed at which symptoms appeared.

Barker (1957) also found that the post-treatment temperature coefficient of DDT (in DDT-resistant house flies) was apparently positive below 15° C. when the flies were counted 24 hr. after treatment, but not when the same flies were counted again 48 hr. after treatment. The reaction of the flies below 15° C. was slower than at higher temperatures. (It may be noted in passing that, in contrast, the positive coefficient found above 30° C. on flour beetles by Pradhan & Rangarao (1957) increased slightly in size as time passed.)

Thus it seems likely that a very low post-treatment temperature had little effect on penetration (stage (a)) or transport (stage (b)), but that it greatly decreased the speed of the chemical or physical process which actually caused paralysis (stage (c)).

I wish to acknowledge my warmest gratitude to Dr A. H. McIntosh for his guidance, inspiration and encouragement throughout the course of this work. I am also very much indebted to Dr C. Potter for his suggestions and constructive criticism of this work. Finally, I am grateful to Mr J. H. A. Dunwoody for his help with the probit calculations.

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The relationships between increased oxygen uptake and locomotor ataxy or death in insecticide-poisoned *Alphitobius laevigatus* F.

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(Received 8 April 1960)

SUMMARY

The relationships between increased oxygen uptake and locomotor ataxy or death induced by LD₅₀'s of *n*-valone (2-*n*-valeryl-1,3-indandione), γ -BHC, *p,p'*-DDT, dieldrin, DNBP (2,4-dinitro-6-*sec*-butylphenol), malathion and allethrin, in individual lesser mealworm beetles (*Alphitobius laevigatus* F.) have been investigated. *n*-Valone did not increase the rate of oxygen uptake of treated beetles. After dosage with DDT or malathion, increased oxygen uptake occurred only in beetles which were knocked down and beetles knocked down always died. After dieldrin treatment, increased oxygen uptake was always associated with knock-down, but a few knocked-down beetles recovered. γ -BHC, allethrin or DNBP caused knock-down or serious locomotor ataxy and increased oxygen uptake in all the treated beetles, but those which eventually died reached higher peaks in rate of oxygen uptake. The similarities and differences between the effects of the various insecticides are discussed together with some aspects of their possible mode of action.

INTRODUCTION

A substantial amount of information is available about the effects of insecticides on the oxygen consumption of insects. Reviews by Edwards (1953) and Metcalf (1955) refer to most of the information, and Lord (1950) and Harvey & Brown (1951) made particularly extensive studies with numerous insecticides. The broad conclusions from the different investigations, with different insect species, agree well. Many insecticides, for example nicotine, pyrethrins, substituted 4,6-dinitro-phenols, DDT, γ -BHC, organophosphorus compounds, and azobenzene, greatly increased the rate of oxygen consumption. Others, for example phenothiazine, rotenone, and organic thiocyanates, depressed it.

However, the relation between the increase in oxygen uptake induced by insecticides and the onset of other symptoms such as locomotor ataxy remains obscure; and whether the effect of an insecticide on the uptake is connected with the eventual death or survival of the insect in general remains unknown. Lord (1950) measured oxygen uptakes of flour beetles, *Tribolium castaneum* Hbst. treated with insecticidal

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dusts, and noted that the onset of increased oxygen uptake induced by γ -BHC or high doses of pyrethrins appeared to coincide in time with the first visible signs of poisoning. However, since he made measurements on groups of beetles, he could not definitely relate the uptake of individuals to the other symptoms. Harvey & Brown (1951) measured the uptakes of individual cockroaches, *Blattella germanica* L., injected with insecticidal solutions, and their results suggest that an increase in oxygen uptake often occurs about the same time as the onset of locomotor ataxy. However, all the cockroaches for which they record results were killed, and no information was obtained about surviving insects. Buck & Keister (1949) and Fulmer & Hoskins (1951) also mentioned a possible connexion between increases in oxygen uptake and locomotor ataxy, but made no special investigation of the subject. Ouye (1959) found that among house-flies topically treated with malathion, only those individuals which received a lethal dose were knocked down and showed oxygen uptakes above normal.

In the present work the relation between locomotor response and oxygen uptake was investigated in greater detail than hitherto, using insecticides representing a wide range of chemical structures applied at doses about equal to the respective LD₅₀'s. Differences were sought in oxygen uptake between individuals killed by an insecticide and those not killed.

MATERIALS AND METHODS

Test insects

The test insects were lesser mealworm beetles, *Alphitobius laevigatus* F., bred at 25° C. on damp wheatfeed by a standardized method (Hewlett, P. S., unpublished), and 1–3 weeks old when treated with insecticide.

Insecticides

The insecticides were pure *n*-valone(2-*n*-valeryl-1,3-indandione), γ -BHC, *p,p'*-DDT, dieldrin and DNBP (2,4-dinitro-6-*sec.*-butylphenol); a 95% malathion concentrate; and a mixture of allethrin isomers obtained by distilling a commercial sample. They were applied to the beetles in solution in Shell Risella oil 17 (R17).

Respirometers

A set of ten respirometers were used for the measurement of the oxygen uptakes of individual beetles. The principle of these respirometers was similar to that described by Fenn (1927). Each respirometer chamber (Fig. 1) was a short tube of about 7 ml. capacity. The insect was housed in the top of the chamber above a disc of perforated zinc, held in place by a spring clip. In the bottom of the chamber was put 0.5 ml. of potassium hydroxide solution and a small roll of Whatman no. 40 filter-paper. The potassium hydroxide solution absorbed carbon dioxide, and kept the relative humidity at $70 \pm 2\%$ (see Solomon, 1951). In a few experiments, in which the beetles respired very rapidly, absorption of carbon dioxide reduced the strength of the potassium hydroxide solution sufficiently to raise the relative humidity to $72 \pm 2\%$ at the end of an experiment. Attached to a side arm was a 1 ml. graduated pipette, calibrated to be read to the nearest microlitre. At the start of an experiment a seal of dyed R17 (oil)

beetles treated with γ -BHC were not kept in the respirometers for more than 6 hr., because the toxic effect of the γ -BHC was shown to be intensified after this period, presumably by its fumigant action.

Where the oxygen consumption of insecticide-treated beetles was very high, the oxygen tension in some chambers fell to about 10%. This concerned only a few beetles treated with DDT or dieldrin, and since the peak rates of oxygen uptake attained by beetles in an oxygen tension of about 10% were similar to those attained by beetles in oxygen tensions of 15% or more, it was considered that the oxygen uptake of the beetles was unlikely to have been seriously affected by oxygen lack.

RESULTS

Preliminary toxicity tests, with groups of twenty beetles, each dosed with 0.03 μ l. of oil solution, gave the following approximate LC₅₀'s: *n*-valone 10% (w/v); γ -BHC 0.6% (w/v); allethrin 5% (w/v); DNBP 2% (w/v); malathion 0.35% (w/v); DDT 2% (w/v); dieldrin 0.3% (w/v). These concentrations were used in the respiration tests.

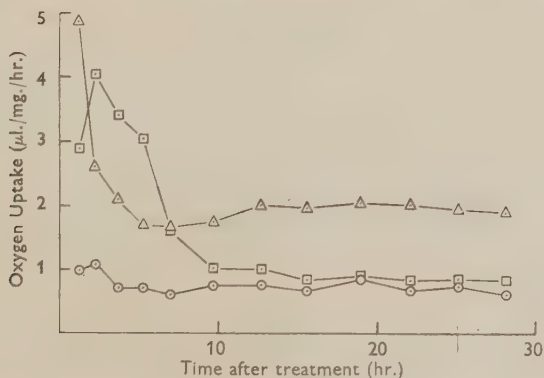


Fig. 2. Oxygen uptakes of single *A. laevigatus* beetles treated with 5% allethrin in oil.
 Δ, Died; □, survived; ○, control.

Experiments with untreated beetles showed that although there was a tendency for females to consume more oxygen than males, the difference was not statistically significant. Also there was no significant difference between the oxygen uptakes of beetles (unsexed) treated with 0.03 μ l. of R17, and untreated beetles. Hence it was considered unnecessary to sex beetles for test, or to treat control beetles with oil. However, when the weights and oxygen uptakes of eighteen untreated beetles were compared over a period of 24 hr., the two were found to be positively correlated ($r = 0.71$, $P = 0.001$). Beetles were therefore weighed before enclosure in the respiration chambers, and their rates of uptake calculated as μ l. of oxygen (corrected to 760 mm. of mercury) per mg. of initial body weight per hour.

Figs. 2-4 are typical graphs of oxygen uptake by control beetles and by beetles which

eventually died or survived after dosage with insecticide. The times at which knock-down was first observed are indicated in Figs. 3 and 4, but not in Fig. 2, because allethrin-treated beetles were knocked down before they were put into the chambers. Owing to the respective lack of respiratory response and short observation period, it

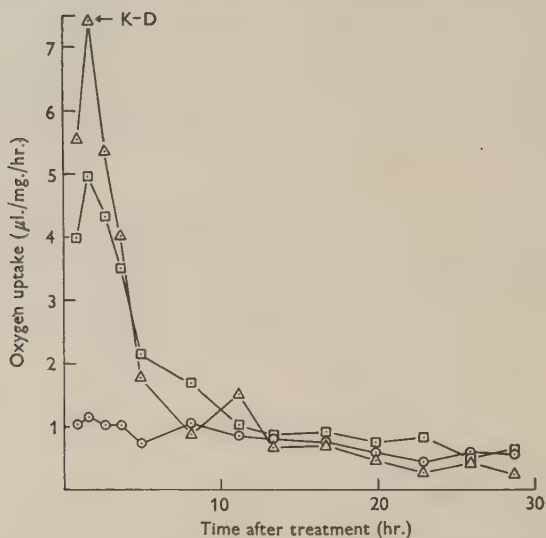


Fig. 3. Oxygen uptakes of single *A. laevigatus* beetles treated with 2% DNBP in oil. Δ , Died; $\square$, survived; $\circ$, control.

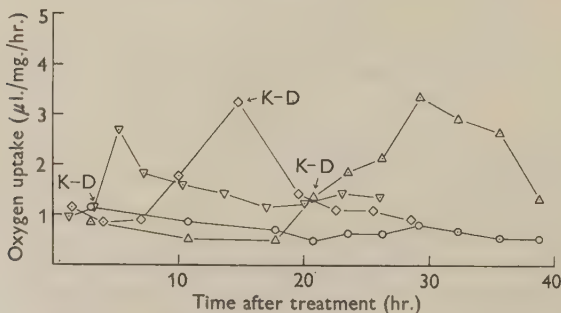


Fig. 4. Oxygen uptakes of single *A. laevigatus* beetles treated with lethal doses of 0.3% dieldrin, 2% DDT, or 0.35% malathion in oil. Δ , Dieldrin; $\diamond$, DDT; ∇ , malathion; $\circ$, control.

was not considered necessary to give respiration graphs for *n*-valone and γ -BHC. Sixteen beetles were treated with each insecticide (eight with *n*-valone), and the ranges and averages of maximum rates of oxygen uptake during the periods indicated in Figs. 2-4 are shown in Fig. 5.

In the following account of the action of different insecticides, some insecticides are grouped together because of certain similarities in their locomotor and/or respiratory effects.

n-Valone. Although only about half were eventually killed, nearly all the treated beetles were knocked down within 2–3 hr. of dosage. However, beetles which eventually survived showed no change in rate of oxygen uptake, and the only apparent change in the oxygen uptake of beetles that had received lethal doses was a steady fall in the rates of uptake of moribund ones. Since *n*-valone did not prove to be a respiratory stimulant it will not be considered further in this paper.

γ -BHC, allethrin and DNBP. All the beetles treated with γ -BHC, allethrin or DNBP showed locomotor ataxy (although, as expected, only about half were killed). All the

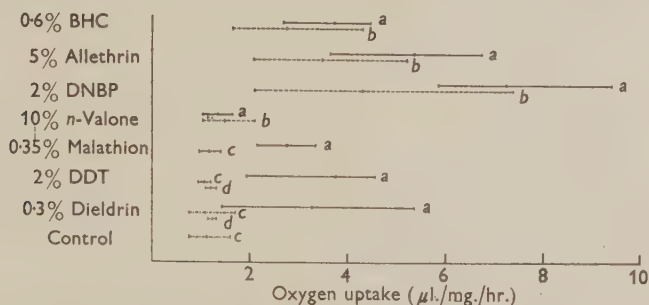


Fig. 5. The ranges, and averages, of maximum rates of oxygen uptake attained by single *A. laevigatus* beetles after treatment with various insecticides. *a*, Died; *b*, recovered after a period of locomotor ataxy; *c*, unaffected; *d*, died (knocked down after period of respiration measurement).

γ -BHC-treated beetles were knocked down within about 5 hr. (most within 2 hr.) after dosage, and all the allethrin-treated beetles were knocked down within about 5 min. Of those treated with DNBP all showed locomotor ataxy, but not all were knocked down. Because beetles dying from DNBP-poisoning became sluggish rather than convulsive, knock-down was not a reliable indication of locomotor ataxy and some beetles eventually died in the prone position.

All the beetles treated with γ -BHC, allethrin or DNBP showed increased rates of oxygen uptake. Possibly some at least of the γ -BHC-treated beetles reached their peak oxygen uptake after the 6 hr. period in the chambers, but it was clear that the beetles were usually knocked down some time before reaching their peak oxygen uptake. Allethrin-treated beetles reached their peak uptake within about 2 hr. of dosage, and therefore, like γ -BHC-treated beetles, after knock-down. The length of time for which the oxygen uptake of beetles eventually killed by allethrin remained above control level, varied from about 10 to 24 hr. or more. DNBP-treated beetles appeared to reach their peak oxygen uptake before serious locomotor ataxy was obvious but, as indicated previously, it was often difficult to assess the latter accurately. γ -BHC, allethrin and DNBP all induced significantly higher maximum rates of oxygen uptake

in beetles which eventually died, than in those which survived. Comparison by *t*-test of average maximum rates of oxygen uptake of dying and surviving beetles gave the following probabilities: γ -BHC, $P = 0.02$; allethrin, $P = 0.002$; DNBP, $P = 0.0015$. However, the average rates of oxygen uptake over the first 6 hr. (γ -BHC) or 20 hr. (allethrin and DNBP) showed that although on average beetles which eventually died had higher uptakes than those which survived, the differences were not significant (γ -BHC, $P = 0.25$; allethrin, $P = 0.15$; DNBP, $P = 0.08$).

Malathion and DDT. Only beetles which later died were knocked down and only these showed an increase in rate of oxygen uptake. Knock-down always occurred during the period of increasing rate of uptake before the peak was reached. The results with malathion therefore agree with those of Ouye (1959). Some DDT-treated beetles were not knocked down until 4 days after dosage, and beetles not knocked down until after the period of respiration measurement did not show any increase in rate of oxygen uptake during the period (Fig. 5).

Dieldrin. This had the slowest action of any of the insecticides tested, and beetles were knocked down up to 5 days after dosage. A small proportion of knocked-down beetles subsequently recovered, especially among those knocked down 2–5 days after dosage. Therefore, although measurements of oxygen uptake during the first 45 hr. after dosage showed that knock-down and increased rate of oxygen uptake were always associated in a manner similar to that found after dosage with DDT or malathion, an additional series of respiration measurements were made, on beetles knocked down 46–110 hr. after dosage, to show whether abnormally high oxygen uptake in dieldrin-treated beetles was associated with knock-down rather than death. Individual beetles knocked down 46–110 hr. after treatment with dieldrin were taken 0–8 hr. after each was knocked down, and their rates of oxygen uptake were measured over the next 16 hr. In eleven individuals that died, the mean rates of uptake in the 16 hr. period ranged from 2.1 to 4.4 $\mu\text{l./mg./hr.}$; in the six surviving individuals they ranged from 2.1 to 3.6 $\mu\text{l./mg./hr.}$ The uptake of control beetles was less than 0.8 $\mu\text{l./mg./hr.}$ Comparison, by *t*-test, showed that the mean oxygen uptakes of dying and surviving knocked-down beetles were not significantly different ($P = 0.4$). This does not, however, preclude the possibility that dying beetles may have reached peaks in uptake significantly higher than those of surviving beetles.

DISCUSSION

Malathion, DDT, dieldrin, γ -BHC, allethrin and DNBP each caused increases in rates of oxygen uptake of treated beetles, and certain conclusions can be reached about the relationships existing between these increases: and (1) the occurrence of locomotor ataxy, (2) the time of onset of locomotor ataxy, and (3) the eventual death or survival of individual beetles.

(1) *Occurrence of locomotor ataxy.* After treatment with malathion, DDT, or dieldrin, only those beetles which were knocked down showed increased rates of oxygen uptake. However, treatment with γ -BHC, allethrin or DNBP induced locomotor ataxy in all the treated beetles, either knocking them down (γ -BHC or allethrin) or severely affecting locomotor activity (DNBP), and all the treated beetles showed

increased oxygen uptake. Therefore, it was not possible to reach a conclusion about any relationship between occurrence of locomotor ataxy and occurrence of oxygen uptake after treatment with γ -BHC, allethrin or DNBP.

(2) *Time of onset of locomotor ataxy.* After treatment with any of the respiratory stimulants tested, except possibly DNBP, the time at which the rate of oxygen uptake began to rise above the control level was always closely linked with the time of onset of locomotor ataxy, and knock-down always occurred at or before the time at which the peak rate of oxygen uptake was reached. After treatment with DNBP large increases in rate of oxygen uptake often occurred before locomotor ataxy could be detected, but because detection of locomotor ataxy was difficult, it was not easy to draw definite conclusions from DNBP-poisoned beetles.

(3) *Eventual death or survival of treated beetles.* All the respiratory stimulants tested produced, on average, higher peak rates of oxygen uptake among the beetles that received lethal doses than among those that survived. These differences were statistically significant with all except dieldrin, where, possibly because of experimental technique, only a tendency was demonstrated.

Beetles which survived treatment with malathion or DDT were never knocked down, and their rate of oxygen uptake never rose significantly above the control level. However, the oxygen uptake of beetles that survived treatment with γ -BHC, allethrin or DNBP rose to peak rates intermediate between those reached by dying beetles and the control level. Among the beetles that survived treatment with dieldrin, some showed neither increased oxygen uptake nor locomotor ataxy, and others were knocked down for a period and showed concurrent increased oxygen uptake.

The lack of locomotor and respiratory response among beetles that survived treatment with malathion or DDT suggests that these responses are not directly connected with the primary toxic action of these insecticides, although the reverse is not necessarily true for the other insecticides tested. Heslop & Ray (1960) induced increased oxygen uptake, paralysis and death in cockroaches that were tied down for long periods and thereby subjected to prolonged physical stress. They suggested that the responses induced in tied-down cockroaches could be regarded as a stress syndrome, and that such a syndrome might be superimposed on the specific response to any convulsant insecticide, the toxic action of which was not very rapid. This explanation could account for the respiratory response of *A. laevigatus* beetles to treatment with malathion or DDT, but since the cockroaches tied down by Heslop & Ray never recovered after a stress syndrome, it does not so easily account for the response of *A. laevigatus* to dieldrin, in which some beetles recovered after a period of increased oxygen uptake and knock-down.

Although in this work, whenever increased oxygen uptake was found, locomotor ataxy appeared to be associated with it, locomotor hyperactivity, of the order of that which often occurs in flies or cockroaches treated with nerve poisons, was not observed. It seemed that such locomotor activity as did exist could not account for all the increases in oxygen uptake found after treatment with allethrin or DNBP.

It is not surprising that generalizations cannot be made about the effects of insecticides of such diverse chemical structure as those used here. Full interpretation of the

relationships shown must await a more detailed knowledge of the modes of action of these insecticides.

I wish to thank Dr P. S. Hewlett for advice given throughout the course of the work, and Dr E. A. Parkin for constructive criticism of the manuscript.

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Observations on the pollination of red clover by bees

I. The yield of seed in relation to the numbers and kinds of pollinators

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(Received 29 February 1960)

SUMMARY

The pollinators of red clover are described and the relationship between the number of seeds set per head and the yield of clover seed is recorded.

Two methods of recording insect pollination in relation to the numbers of seed set per head of clover were explored in 1955. In 1956 and 1957 recording of actual crops of seed was continued.

Certain species of humble-bees are important in the production of singlecut red clover seed, and in the production of doublecut red clover seed in 1956, although another factor, possibly honey-bees, was dominant in 1957.

There was no indication that 'robbing' was associated with a lower 'seed set'; on the contrary in 1957 it was associated with a higher 'set', possibly because it attracted honey-bees to the crop.

The data showed that the numbers of the different species of humble-bees varied, but gave no indication of the relative effectiveness of the various species in pollination. Those present in greatest numbers were of most value for pollinating red clover.

Pests and diseases likely to reduce seed set were found to be negligible during 1955, 1956 and 1957. Differences in fertility between one field and another were small.

INTRODUCTION

The controversy on the importance of humble-bees in the production of red clover seed is of long standing (Plath, 1934). Referring to self-fertility in red clover, Williams (1925) states: 'Red clover is appreciably more self-fertile in the United States and in Canada than in Britain. In this country the crop may be regarded for all practical purposes as completely self-sterile; cross-pollination is mainly effected through the agency of a comparatively small number of species of humble-bees, assisted to a very slight extent in dry summers by honey-bees.' Bird (1944) concluded from a 5-year study of seed setting in doublecut red clover in North America that the number of humble-bee visitors was much more important in seed setting than the number of honey-bee visitors or the number of florets per head. Stapel (1935) and Pedersen (1935) in Denmark, Wexelsen (1935) in Norway, Åkerberg (1952) and Carlsson (1945) in Sweden related the low yields of clover seed to lack of insect pollination. Butler, Free & Simpson (1956) referred to the difficulties and uncertainty of attempts to

increase visits to red clover by bees and suggested surveys of commercial crops to determine whether insufficient pollination was an important cause of poor red clover seed yields. Hawkins (1958) found a significant negative correlation between size of field and numbers of seed per head in red clover crops in 1954, 1955 and 1956, which suggested that lack of pollination might be a cause of low seed set.

Butler (1941) made records of the pollination of red clover in 1939 and 1940, but very few data have been collected on numbers and kinds of insects pollinating red clover in Britain.

SPECIES OF POLLINATORS RECORDED

The species of humble-bees were identified by reference to Sladen (1912). No attempt was made to distinguish between certain species which, according to Richards (1927), are included in the same subgenus. The pollinators observed on red clover were grouped as follows:

Agrobombus Vogt. Four species included in this subgenus were observed: *Bombus agrorum* Fabricius, *B. ruderarius* Müller (*B. derhammellus* Kirby), *B. sylvarum* Linnaeus and *B. solstitialis* Pz (*B. helferanus* Seidl.). An attempt was made to record these species separately, but their behaviour was similar and *B. agrorum* was the most numerous.

Hortobombus Vogt included *B. hortorum* L. and *B. ruderatus* Fab. which were similar in appearance and in behaviour as pollinators of red clover. No attempt was made to distinguish between these species and *B. subterraneus* L. (*B. latreillellus* Kirby), which has similar external marking although belonging to another subgenus.

Lapidariobombus Vogt was represented by *B. lapidarius* L.

Bombus Latreille (*Terrestribombus* Vogt) included *B. terrestris* L. and *B. lucorum* L. which were similar in appearance and behaviour.

Apis mellifera.

CHARACTERISTICS OF POLLINATING AND OF 'ROBBER' BEES

The characteristic appearance of a long-tongued bee (*B. hortorum* L.) visiting a red clover flower is shown in Fig. 1A. Another black bee with buff markings (*B. lucorum* L.) is shown in Fig. 1B. Not only can these bees be readily distinguished in the field by the presence of a second thoracic buff band in *B. hortorum* but also by its longer and narrower shape. Its behaviour, standing erect on the flower with tongue extended, is characteristic of a 'positive' pollinator. The second bee is a 'robber' and is seen creeping between the florets to obtain nectar through a hole in the corolla tube. These 'negative' pollinators do not fertilize the floret and were recorded separately. The posture of a 'positive' honey-bee is shown in Fig. 1C, while 1D shows a 'negative' honey-bee 'robbing' nectar from a floret. These distinctive characters made it possible to record the kinds of pollinators observed in the field and their behaviour, whether 'positive' or 'negative'.

YIELD OF SEED AND SEED SET

'Seed set', as indicated by the average number of seeds per ripe head of clover, is usually closely correlated with the quantity of seed harvested (Hawkins, 1956, 1958).

However, in the wet year of 1956 the correlation was not significant probably because of excessive losses in harvesting the seed.

Seed set was determined by counting the number of seeds in five samples each of twenty ripe heads of clover and was expressed as the mean number of seeds per head. Small labels were attached at weekly intervals to a number of heads on which half the

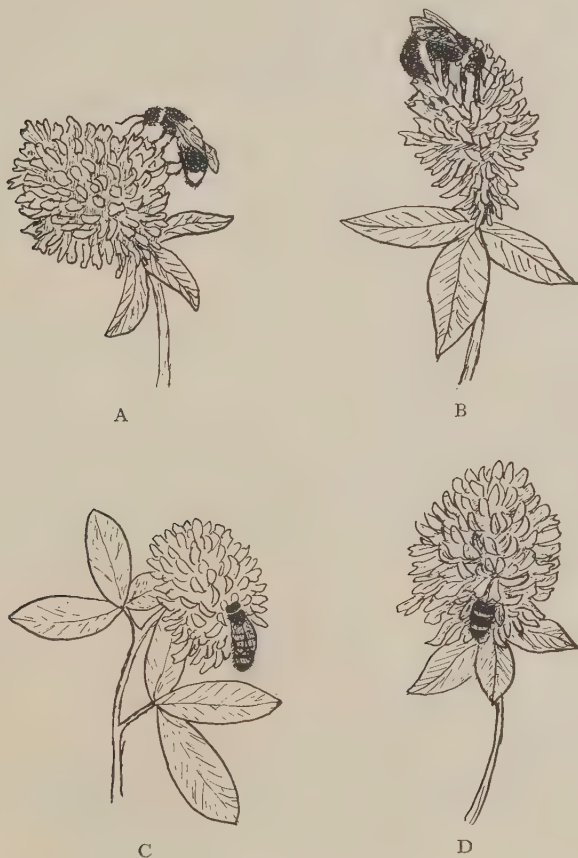


Fig. 1. A, *B. hortorum* L.; B, *B. lucorum* L.; C, honey-bee pollinating; D, honey-bee robbing.

florets were open. These indicator heads were closely watched and when those labelled on the same day had turned brown, but with the stem still green, a head sample was collected, each head being in the same stage of ripeness as the indicator heads. By this means the approximate time was recorded when each head sample was flowering.

Pests and diseases

A low seed set could result from an attack of clover seed weevil (*Apion* sp.) or clover midge (*Dasyneura* sp.) or from the pollen being destroyed by pollen mould (*Botrytis* sp.).

Heath (1955, 1956) records that weevil and midge infestation was negligible in both years. In 1957 clover head samples were examined for weevil as described by Jones (1950), and for midge by detector cages (Barnes, 1954). Very few weevils and midges were found in 1957 and only a trace of pollen mould (*Botrytis* sp.) occurred in each of the three years under review.

Soil fertility

Leaf samples were collected from all crops and analysed to determine the mineral status of the crop. Differences were small and unlikely to affect the seed in those crops included in the investigation. One of the crops for observation in 1955 was discarded, as it was situated on a pocket of gravel and died from drought soon after flowering.

PREVIOUS METHODS OF RECORDING POLLINATORS VISITING RED CLOVER

Carlsson (1945) confined honey-bees within cages on a crop of clover, but found that the bees so confined crawled round the walls and died. Morrison (1942) visited plots of red clover at 11 a.m. and 4 p.m. on 3 days each week and walked round the plots for a period of 20 min. on each occasion, recording the number and species of each bee observed. Stapel (1934) counted the number of bees on an area of 6×167 m. (1002 m.^2) but preferred an area of 500 or 250 m.^2 with a width of 2.5 for recording honey-bees. Pedersen (1935) counted the number of bees on a marked area of 300 to 400 m.^2 on each field every second or third day while the crop was flowering and recorded the species and method of working.

METHODS FOLLOWED IN THESE INVESTIGATIONS

In 1955 two methods of recording pollinators in relation to seed set were explored.

Method no. 1. A series of thirty-two plots of singlecut and late-flowering red clover 30 ft. by 5 ft. had been sown broadcast the previous year on heavy land at N.I.A.B. Some of these plots were cut before flowering in 1955 so that a succession of flowers was available. The numbers and kinds of bees visiting these flowers on eight plots 5 ft. by 7 ft. were recorded during a period of 20 min. at 11 a.m. and 4 p.m. on 3 days a week. Heads were labelled each week, collected when ripe and the number of seeds counted.

Humble-bees were very numerous in the field where these observations were made, but the results were disappointing, for the correlations between seed set and numbers of pollinators were not significant.

Method no. 2. In the second method tried in 1955, records were made on six fields of singlecut red clover growing within a few miles of one another so that all crops could be visited and records made within a reasonably short time. The order of visiting the crops was at random as also was the time of day. The numbers and kinds of pollinators were recorded on five plots 11 yards by 5 yards set out round three sides of a square on

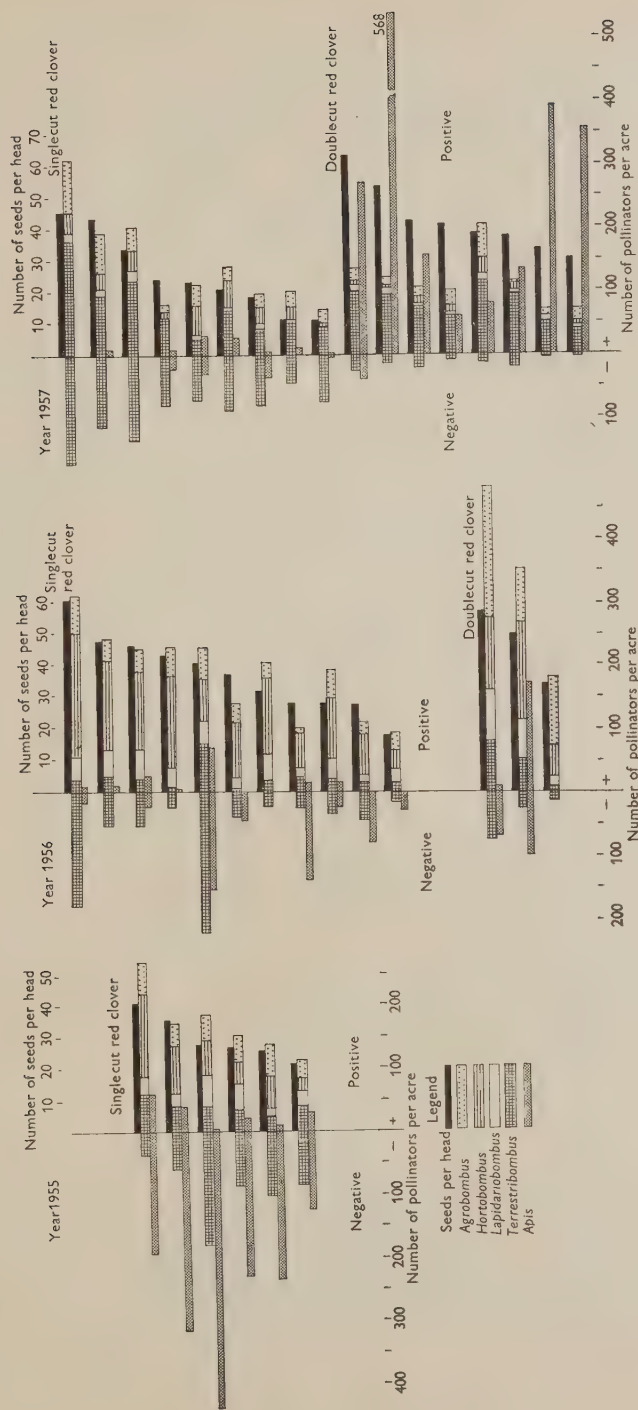


Fig. 2. Number of seeds per head and numbers of positive and negative pollinators in crops of singlecut and doublecut red clover in 1955, 1956 and 1957.

Table 1. Coefficient of correlation r between 'seed set' in singlet and doublet red clover and pollinators in the east of England and coefficient of regression b of 'seed set' on numbers of pollinators per acre during 1955, 1956 and 1957

	Singlet				Doublet			
	1955		1956		1957		1956	
	r	b	r	b	r	b	r	b
Positive pollinators	0.9310**	0.124	0.8180**	0.121	0.8831**	0.150	0.9259	0.3208
Negative pollinators	-0.0560	—	0.3516	—	0.7934*	0.288	0.9846	0.7472*
Positive humble-bees	0.9002*	0.148	0.8898**	0.145	0.8778**	0.143	0.9901*	0.2641
<i>Agrobombus</i> spp.	0.3572	—	0.6492*	0.515	0.8240**	0.448	0.6237	-0.0963
<i>Hortobombus</i> spp.	0.9524**	0.179	0.8856**	0.222	0.3534	—	0.7825	-0.0637
<i>B. lapidarius</i>	-0.0220	—	0.6230*	0.624	0.7292*	1.680	0.9997*	0.2677
<i>Terrestrisbombus</i> spp.	-0.3502	—	0.4988	—	0.8531**	0.127	0.9514	0.5611
Honey-bees	0.0617	—	-0.0114	—	-0.2830	—	0.4924	0.3059
Number of crops	6		11		9		3	7
Mean number of seeds per head	29.5		37.1		25.5		46.9	42.2

Significance at $P = 0.05$ is indicated by * and at 0.01 by **.

one side of each field; a total of 275 square yards. The date, time, weather and the duration of the observations were recorded at each visit. The visits started when the crop was just coming into flower and continued three times weekly for a period of 4 or 5 weeks until most of the flowers had withered.

A direct comparison cannot be made between the number of pollinators recorded by the two methods, for by the first method the basis of comparison was the number of pollinators recorded during a definite period of time, while in the second method the number of pollinators on a measured area was recorded. Nevertheless, an estimate was made of the area covered by the first method and on this basis there were nearly three times as many pollinators as there were on the commercial crops which were recorded by the second method.

Observations on six crops of singlecut in East Hertfordshire in 1955 established a relationship between pollinators and seed set as noted by Hawkins (1956). It was decided, therefore, to use method 2 for the rest of the investigations. It was found that the difference between records made by two experienced observers on the same crop at the same time was less than that made by the same observer on the same crop on different days in the same week; hence two observers were used in 1956 and 1957. In 1956 observations were made on three crops of doublecut red clover and eleven crops of singlecut in east Cambridgeshire and Hertfordshire. In 1957 four crops of singlecut in east Hertfordshire and five in Cambridgeshire were recorded, together with eight crops of doublecut red clover in the same districts.

In 1955 it was necessary to catch a number of pollinators to identify them and the time taken at each crop was between 20 and 30 min., but in 1956 and 1957, when the appearance of different pollinators was known, the time was reduced to 10–15 min. at each visit.

The mean number of seeds per head and the numbers and kinds of pollinators are given in Fig. 2, showing the mean number of seeds per head from each crop, the number of humble-bees per acre, divided according to the numbers of the different subgenera, and the number of honey-bees per acre. The numbers of negative pollinators obtaining nectar through a hole in the side of the corolla tube are distinguished from those of the positive pollinators. Table 1 gives the mean set seed each year, and the coefficient of correlation between 'seed set' and the numbers of pollinators of different kinds. Where this is significant or approaching significance the regression of number of seeds per head on numbers of pollinators is also given.

DISCUSSION

Numbers of pollinators and seed set

The investigations into the factors affecting the yield of red clover seed were specifically concerned with the yield of late-flowering red clover; but there were so few crops of this type grown near Cambridge that observations were made on singlecut red clover, which flowers between true late-flowering and doublecut red clover. Plots of true late-flowering red clover were represented at the Institute by Aberystwyth S. 123,

Montgomery late and Cornish Marl. A considerable acreage of doublecut red clover is grown around Cambridge and crops of this, as well as singlecut, were included in 1956 and 1957 to provide a more complete picture of pollinator activity in the east of England.

As shown in Table 1 there was a close correlation each year between 'seed set' in singlecut red clover and the number of 'positive' humble-bees recorded. The influence of other factors likely to affect 'seed set'—soil fertility, pests and diseases—was found to be negligible so that it can be concluded that in 1955, 1956 and 1957 the number of positive humble-bees visiting these crops was a major factor in affecting 'seed set'. Moreover, the mean number of seeds per head on these crops was low compared with that on some crops in 1955. The data indicate that the low 'seed set' found in the series of singlecut red clover crops examined in 1955, 1956 and 1957 was due to insufficient numbers of positive humble-bees.

The data on numbers of pollinators visiting doublecut red clover are more limited but they are of interest. In both 1956 and 1957 the heads had a higher 'seed set' than in the crops of singlecut. There was a close correlation between 'seed set' and numbers of positive humble-bees in 1956 but it was not significant in 1957. In that year the only significant correlation was a positive relationship between 'seed set' and numbers of negative pollinators. Moreover, this rather surprising correlation was significant, with others, in the nine crops of singlecut recorded in 1957 and was high, although not significant, in the three crops of doublecut red clover recorded in 1956. It is difficult to explain why negative pollinators should increase 'seed set' unless they are associated with some major factor which has not been recorded. The possibility that this may be honey-bees working the crop for pollen is discussed later.

It will be seen from Fig. 2 that the numbers and kinds of pollinators vary considerably from year to year as well as from crop to crop and the data were examined to see if they indicated the relative importance of different kinds of pollinators.

Value of honey-bees as pollinators of red clover

An attempt was made each year to record the numbers of honey-bees and whether they were positive or negative. As will be seen from Fig. 2 large numbers of honey-bees were observed visiting the crops of singlecut in 1955; nearly all were extracting nectar through a hole in the side of the corolla tube. The correlation between numbers of honey-bees and 'seed set' was quite insignificant; much the same occurred in 1956. One of the crops of doublecut recorded in 1956 was visited by a large number of honey-bees for a short period, but the correlation between 'seed set' and numbers of honey-bees was not high and failed to reach significance. In 1957 there were very few honey-bees recorded on the singlecut crops but there were large numbers on many of the crops of doublecut, practically all of which were effective pollinators and yet the correlation between 'seed set' and numbers of honey-bees was quite insignificant.

The positive correlation which occurred in 1957 between 'seed set' and numbers of negative pollinators is of particular interest for it supports the view expressed by Free & Butler (1959) that *Terrestrisbombus* spp. by piercing holes in the side of corolla

tubes and thus making the nectar accessible to honey-bees might in fact be furthering pollination. The honey-bees attracted to the crop might also collect pollen from it and in so doing effectively pollinate the flowers. Schwan (1953) has compared the numbers of humble-bees and the numbers of honey-bees visiting a crop of red clover at different times during the day on four different days. The variation in numbers of honey-bees on different days and at different times during the same day is very great compared with the corresponding variation in numbers of humble-bees. The methods which have proved effective in recording the numbers of humble-bees may not give a true picture of the numbers of honey-bees. There is little reason for assuming, however, that honey-bees made any contribution to the pollination of singlecut red clover apart from 1957, for in the other years the correlation between 'seed set' and both negative pollinators and honey-bees was quite insignificant.

The effect of 'robbing' on 'seed set'

The frequency with which *Terrestrisbombus* spp. rob red clover flowers has led to much speculation as to the effect on yield of seed. Stapel (1934) suggests that exterminating or suppressing *B. terrestris* would result in an increase in the value of honey-bees in red clover seed production.

Forster & Hadfield (1958) remark that the effectiveness of the humble-bee population in New Zealand is greatly reduced by the large number of *B. terrestris* that bite through the sides of the florets, but nevertheless they are probably equal in value as a pollinator to *B. ruderatus* because of their superior numbers. Williams (1925) refers to robber bees, *B. terrestris* and *B. lucorum*, which were fairly numerous but states that the extent to which yields of clover seed may be reduced has not yet been ascertained.

During the 3 years under review it would seem that apart from the possibility of encouraging honey-bees to visit the crop to collect pollen *B. terrestris* made a direct contribution to the yield of singlecut red clover seed in 1957. Moreover, in 1955 and 1956 when large numbers were robbing, there was no indication from the data that they were associated with lower 'seed set'. From the data collected in this investigation there is no evidence that *Terrestrisbombus* spp. are in any way detrimental to the production of red clover seed in the east of England and there were occasions when they were distinctly beneficial.

The value of different species as pollinators

Stapel (1933) divided humble-bees and honey-bees into five groups according to the number of florets pollinated per minute. The present data have been examined, therefore, to see whether there is any indication that certain species are more efficient as pollinators than others.

In Table 1 it will be noted that where the coefficient of correlation is significant, the coefficient of regression has been examined. There was a significant correlation each year between 'seed set' in crops of singlecut and the numbers of positive pollinators; the coefficient of regression varied between 0.121 and 0.150 which agrees very closely with

that for positive humble-bees. The coefficient of regression of *Hortobombus* spp. on 'seed set' for 1955 and 1956 was 0.179 and 0.222 and for *Terrestrisbombus* spp. for 1957, 0.127. On the other hand, that for *B. lapidarius* for 1956 and 1957 and *B. agrorum* for 1957 was considerably higher, although the numbers of bees concerned was quite small. It is doubtful whether any valid conclusions may be drawn from the data regarding the relative efficiency of the different species as pollinators of red clover; but the differences in the numbers of different species present were considerable and the total numbers present are likely to have had an important bearing on the yield of red clover seed.

The author wishes to thank Mr F. R. Horne, Director of the National Institute of Agricultural Botany, for the interest he has taken in this work, and Messrs G. W. G. Briggs and A. F. Kelly for helpful advice in drafting this paper. It is a pleasure to acknowledge the work of Miss M. J. Stokes, now with Messrs Dunns Farm Seeds Ltd., who collected and analysed much of the data, of Miss G. M. Clouting who examined clover heads for weevil and midge in 1957 and Miss J. A. Walker who prepared the line drawings. The examination of clover heads for pollen mould was undertaken by Dr C. C. V. Batts and Dr D. A. Doling.

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Fluctuations in the aerial density of *Anisopus fenestralis* Scop. (Diptera) above sewage bacteria beds

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(Received 21 May 1960)

SUMMARY

Using a standard 9 in. diameter disc-dropping suction trap, the aerial density of *Anisopus fenestralis* above bacteria beds was assessed hourly on 410 24-hourly occasions over a period of 19 months, and the prevailing temperatures and wind velocities were recorded. Between 0 and 10 m.p.h. wind velocity there was a gradual rise in catch associated with a rise in temperature to an optimum, beyond which the catch decreased. This optimum fell as wind velocities rose; it occurred between 70° and 75° F. at winds between 2 and 4 m.p.h., at 65-70° F. with winds between 4 and 6 m.p.h. and at 60-65° F. between 6 and 8 m.p.h. At all temperatures between 30 and 80° F. the catch fell as wind velocity increased from 0 to 10 m.p.h. By comparing the dusk catches on successive days a significant positive correlation was found between the deviation in log catch from a 3-day running mean and the corresponding temperature deviation, except when the wind velocity deviation exceeded +2. There was also a significant negative correlation between deviation in log catch from the running mean and the corresponding deviation in wind velocity, except when the temperature deviation exceeded -2.

The average regressions showed that for each °F. rise in temperature the log catch was raised by 0.14 and for each 1 m.p.h. rise in wind velocity the log catch was decreased by 0.19. Expressed arithmetically the catch was doubled by a 2.2° F. rise in temperature or by a fall in wind velocity of approximately 1.5 m.p.h.

The aerial density was affected not only by weather but also by fluctuations in the *A. fenestralis* population within the bed arising from direct and indirect climatic effects on its food supply—the microbial growths within the bed. Lower winter temperatures favour an accumulation of food, so the patterns of seasonal incidence of the flies from year to year are affected by the severity of the preceding winter.

INTRODUCTION

Flies breeding in sewage bacteria beds, although contributing in their larval stage to the efficiency of the bed by grazing on the biological film, may cause a nuisance on and around the works when they leave the beds as adults. Beds are therefore often treated with insecticides, but this upsets the ecological balance and may result in an undesirable accumulation of film: consequently insecticide treatment is kept to a minimum compatible with the prevention of nuisance.

The work reported in this paper was carried out to determine the factors influencing the aerial density above the beds of *Anisopus fenestralis* Scop., a fly which has caused much nuisance on and around several sewage works.

METHODS

Assessment of aerial density

The population of *Anisopus fenestralis* above the bacteria beds was assessed by a standardized 9 in. flared suction trap kindly loaned by Rothamsted Experimental Station. The trap (see Taylor, 1951), consists essentially of a gauze cone mounted vertically under a 9 in. Vent-Axia fan which draws in air at about 20,000 cu.ft./hr. The flies pass down the cone and are collected in a detachable tube below. To reduce the effect of wind velocity on the air flow through the trap, a flared inlet is fitted and the gauze cone is shielded by fabric (Taylor, 1955). By means of a solenoid-operated release mechanism housed immediately below the fan, a disc is dropped each hour down an axial rod into the collection tube, thus segregating the catch into hourly samples. The discs carry cloth fringes which, before the trap is set, are impregnated with pyrethrum, thus preventing the trapped flies from passing from one hourly sample to another or escaping up into the cone. At the end of the 24 hr. trapping period the collecting tube is removed and the flies trapped each hour are counted.

Although originally designed for aphids, the trap was found suitable for the trapping of *A. fenestralis*—a weak flier about 7 mm. long. *Psychoda* spp. were also trapped in numbers but the larger and more powerful flier *Spaziphora hydromyzina* was rarely taken. The only difficulty experienced in trapping *A. fenestralis* was that when they were very numerous the collecting tube became full, making the segregation of the hourly catches impossible; however, this could be overcome by increasing the length of the collecting tube and the depth of the spacing collars on the discs. The problem of the deposition of an air pollution film on the axial rod, which hindered the free fall of the discs into the collecting tube was solved by the application of 'antiscuffing paste'.

The trap was mounted over the middle of a bacteria bed the surface of which was some 6 ft. above ground level, with the mouth of the trap 4 ft. 6 in. above the surface of the bed.

Meteorological measurements

For each 24 hr. period when the trap was operated, air temperatures were recorded by a thermograph in a Stevenson screen 4 ft. above the bed and 4 yd. from the trap. Average daily wind velocities were calculated from readings on an indicating cup anemometer mounted at the level of the trap inlet some 10 yd. distant.

Usually readings were also taken over a 2 hr. period about dusk. The hours of sunshine as recorded at Edgbaston observatory, some 7 miles south-west of Minworth, were used. The 24 hr. periods for which the trap was operated commenced at 10.00 hr. G.M.T. (9.00 hr. G.M.T. during Summer Time).

RESULTS

(1) *Hourly catches under different climatic conditions*

The 8340 individual hourly catches will first be considered in relation to the corresponding hourly temperatures and mean daily wind velocities. These hourly occasions were divided into fifty-five arbitrary combinations of temperature and wind velocity, the temperature varying in stages of 5° F. from 30° F. to 85° F. and the wind velocity in stages of 2 m.p.h. from 0 m.p.h. to 10 m.p.h. Table 1 gives the average number of *Anisopus fenestralis* trapped under these varying climatic conditions together with the corresponding number of occasions such conditions prevailed.

Table 1. *Average numbers of Anisopus fenestralis taken per hour under different climatic conditions*

		Temperature (° F.)												
		30	35	40	45	50	55	60	65	70	75	80	85	
Wind velocity (m.p.h.)	0	$\bar{x}$ — n 0	— 0	1·8 18	1·5 10	21·2 36	42·1 24	96·3 6	— 0	— 0	— 0	— 0		
	2	$\bar{x}$ 0·06 n 129	0·42 211	0·95 232	4·1 423	20·8 551	38·0 490	77·2 281	113·4 132	133·2 50	17·9 23	— 0		
	4	$\bar{x}$ 0 n 46	0·015 194	0·23 497	2·6 450	10·4 573	21·0 673	32·0 430	44·6 194	29·9 101	5·4 34	1·0 15		
	6	$\bar{x}$ 0 n 49	0·01 61	0·15 189	1·7 392	5·4 487	9·9 337	13·6 179	11·5 63	0·7 4	— 0	— 0		
	8	$\bar{x}$ — n 0	0·2 37	0·04 142	0·3 156	2·8 225	1·6 119	3·5 68	3·4 9	— 0	— 0	— 0		
	10													

$\bar{x}$ = average number of *Anisopus*; n = number of hourly occasions such conditions occurred. The figures in bold are the maximum counts for a given wind velocity.

At all temperatures there was a marked fall off in the average aerial density with increase in wind velocity above 4 m.p.h.: at any one wind velocity the density rose with the temperature up to an optimum temperature above which fly density decreased. This optimum appeared to be lower at higher wind velocities. These results, based on the large numbers of records over a period of nearly 20 months, imply only the coincidence of fly density and certain climatic conditions. No direct relationship in terms of cause and effect is claimed; it may well be that certain climatic conditions occur at certain times of the day or season when, for other causes, the fly density is high.

For each of the fifty-five climatic conditions defined above, the percentage number of occasions when the hourly catch exceeded 10, 50 and 200 respectively were calculated. The results are expressed graphically in Fig. 1, where the fifty-five climatic conditions are represented by the squares and the areas of the contained circles represent the percentage number of occasions when the catch exceeded the stated

values. Thus the unshaded areas represent hourly counts of between 10 and 50, the hatched between 50 and 200, and the black, counts of over 200. It is seen that hourly catches exceeding 10 occurred in thirty of the fifty-five climatic conditions, an arbitrary 10% occurrence figure being exceeded over a temperature range of 45–80° F. and a wind velocity range of 0–8 m.p.h. The decrease in catches with increase in wind velocity is demonstrated at all temperature ranges by the diminishing sizes of the circles from top to bottom of the columns.

Catches exceeding 50 occurred over a narrower range of climatic conditions, and at the 200-catch level these conditions were further restricted, the arbitrary 10% occurrence figure being exceeded only between 60 and 75° F. and at wind velocities less than 4 m.p.h. Again the results, although illustrating the coincidence of fly density and certain climatic conditions, cannot be taken as evidence of a direct functional relationship between the two.

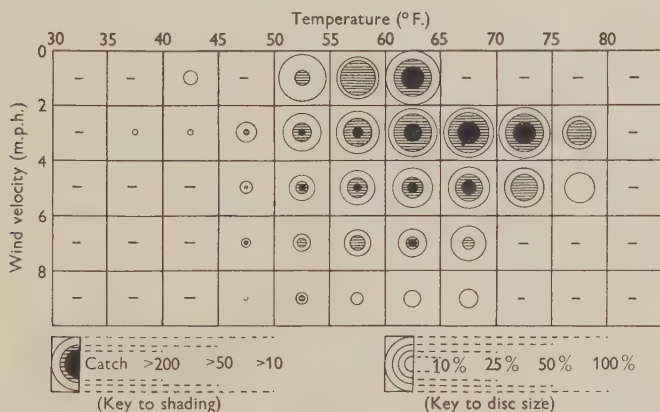


Fig. 1. Percentage number of occasions when catches exceeded 10, 50 and 200 per hour under different climatic conditions.

(2) Relationship between temperature, wind velocity and aerial density

To minimize some of the effects of diurnal variation, demonstrated later, the fluctuation of the fly-population within the bed, and climatic changes such as rainfall and humidity, it was decided to compare day-to-day variations in the catch for the 2 hr. period around dusk with the corresponding variations in temperature and wind velocity. The deviations of the log counts from a 3-day running mean and the deviations in temperature and wind velocity from the 3-day running means were calculated. In all, some 130 sets of deviations were obtained. To compare the effects of temperature these were divided into six ranges of wind velocity deviations. The graphs in which the deviations of the log count from a 3-day running mean (dev. log $\times$ 3-D.R.M.) were plotted against the deviations in temperature (° F.) from the 3-D.R.M. for the different wind velocity ranges are shown in Fig. 2. Except when the

wind velocity deviation exceeded +2 m.p.h. there was found to be a significant positive relationship between deviations in catch and deviations in temperature. A 1° F. change is associated with 0.14 change in the log catch, or the catch is doubled by a

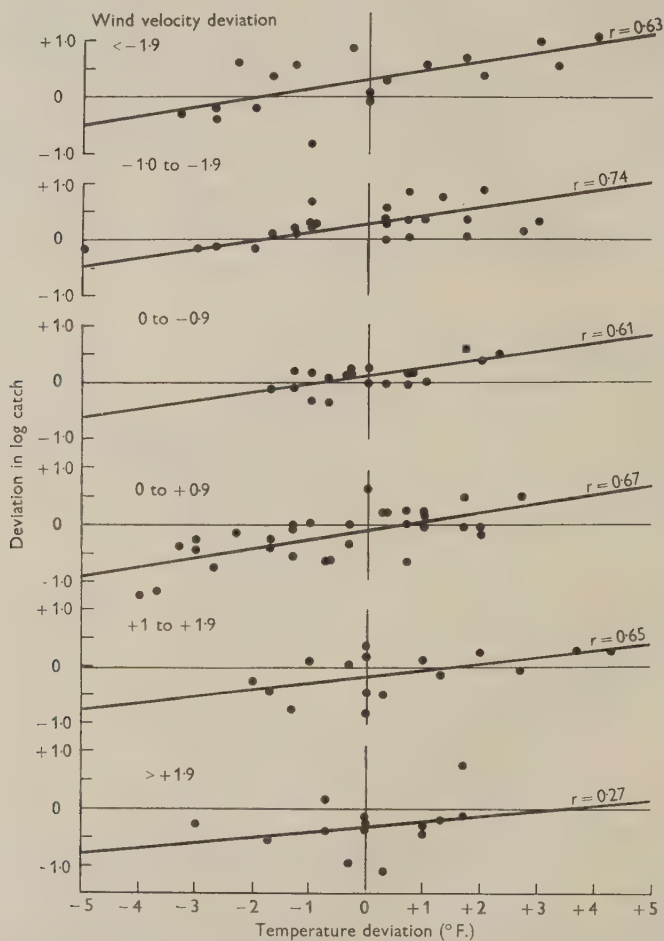


Fig. 2. Regression lines for relation between deviation of log catch and corresponding deviation of temperature for different wind velocity deviation ranges.

2.2° F. rise in temperature. The effect of wind velocity is indicated by the progressive shift in position of the regression line.

To investigate the effect of wind velocity the results were re-arranged into five ranges of temperature deviation, viz. > +2, +1 to +1.9, +0.0 to -0.9, -1.0 to -1.9 and < -2. Regression of deviations of the log catch from a 3-day running mean

(dev. log $\times$ 3-D.R.M.) on corresponding deviations in wind velocity from the 3-D.R.M. for the different temperature deviations is shown in Fig. 3. Except when the temperature deviations exceeded -2° F. a significant negative correlation was found between

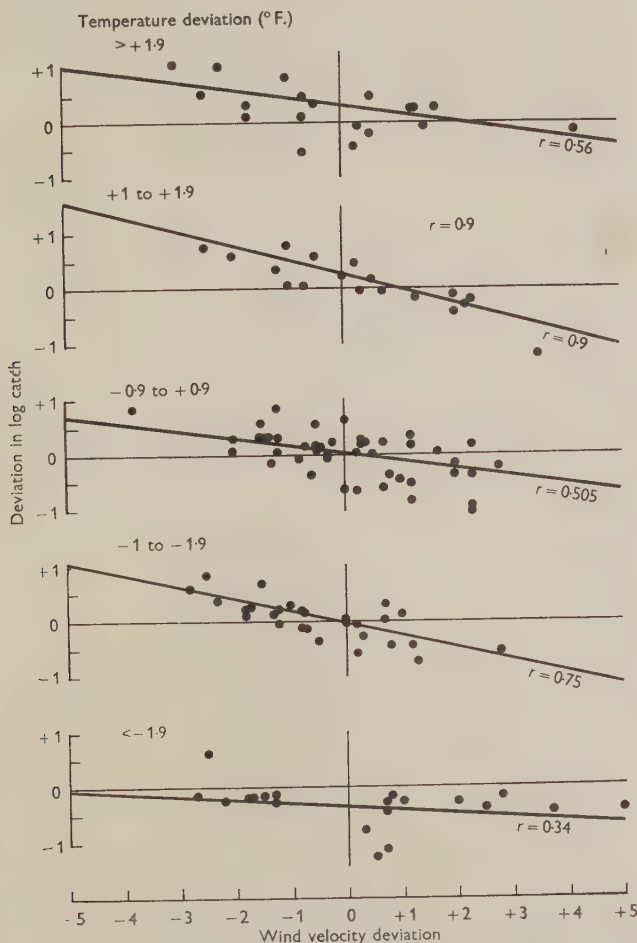


Fig. 3. Regression lines for relation between deviation of log catch and corresponding deviation of wind velocity for different temperature ranges.

the deviation of the log catch from the 3-D.R.M. and the deviation of wind velocity from the 3-D.R.M. A 1 m.p.h. change in wind velocity is associated with a 0.19 change in log catch; or the catch is almost doubled by a fall in wind velocity of 1.5 m.p.h.

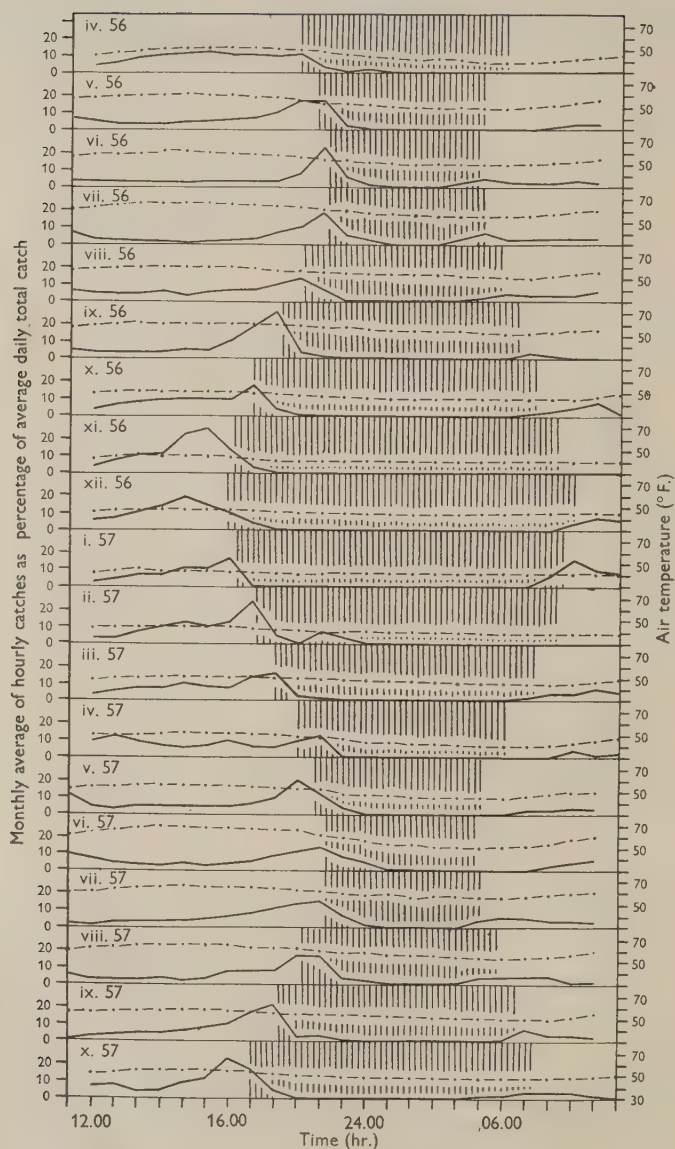


Fig. 4. Diurnal fluctuations in aerial density of *A. fenestralis* in relation to length of day for different months of the year. —, Catch; - - -, temperature; |||, hours of darkness.

(3) *Diurnal fluctuations in aerial density*

Diurnal fluctuations in the aerial density of *A. fenestralis* above the beds, previously observed on isolated occasions (Hawkes, 1952*b*), occurred on most occasions, as shown in Fig. 4, where the monthly average catch for each hour of the day expressed as a percentage of the average daily total is shown graphically for each month. In all months, the flies were much less active during darkness and usually there was a marked increase in aerial density about dusk and sometimes also about dawn.

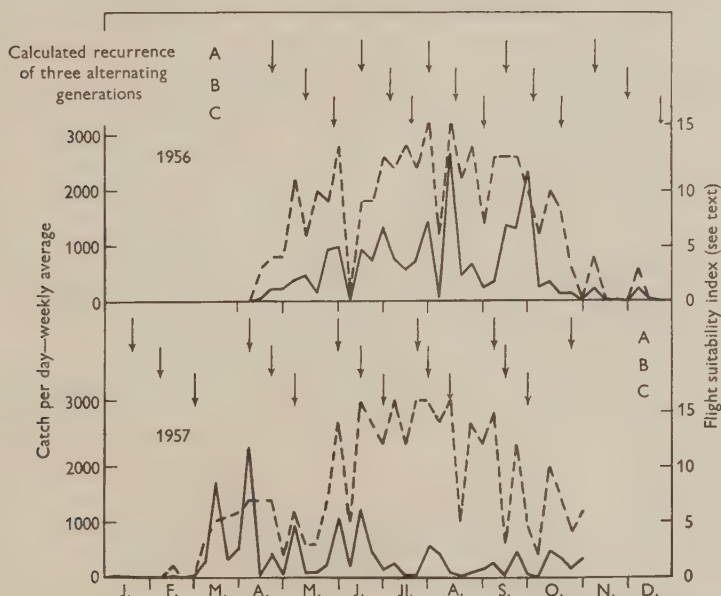


Fig. 5. Weekly and seasonal fluctuations in aerial density above beds and corresponding suitability of climatic conditions for flight. —, Nos. of *Anisopus*; ---, flight suitability.

Although in the winter, night temperatures could themselves reduce activity, a similar reduction occurred in the summer when temperature was not limiting; the dusk increase in activity also often occurred at times of temperature fall: it is therefore probable that this diurnal pattern was associated with light intensity and not with temperature.

(4) *Seasonal fluctuations in aerial density of Anisopus fenestralis*

Fig. 5 shows the weekly average of the daily catches for the 85 weeks of trapping. During 1956 the flies were frequent on several occasions between May and October, few flies being trapped again until March 1957. During 1957, however, the period of

abundance was between March and June with reduced numbers during the remainder of the summer. To find whether these seasonal fluctuations were associated with climatic changes, an assessment of flight suitability was made for each day of trapping. This was calculated from the number of hours favouring flight; i.e. having temperatures between 50 and 75° F. at wind velocities of 0–4 m.p.h., between 55 and 75° F. at wind velocities of 4–6 m.p.h. and between 60 and 70° F. at wind velocities of 6–8 m.p.h. These figures are based on the results expressed in Fig. 2. The weekly average of the daily counts was plotted together with the corresponding flight suitability figure for the whole of the 19 months of observations (Fig. 5). It is seen that marked depressions in the flight suitability graph are usually associated with a

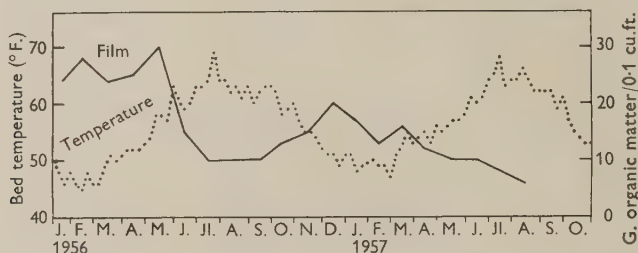


Fig. 6. Comparison of film accumulations in two winters of different severity.

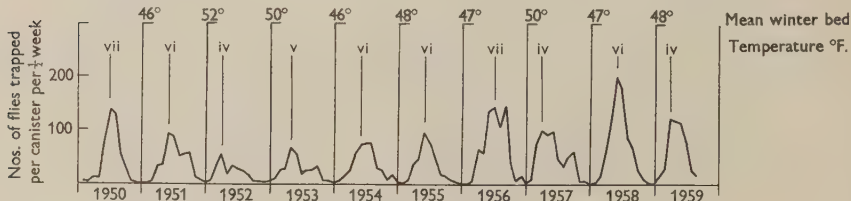


Fig. 7. Seasonal incidence of *A. fenestralis* trapped in canisters above sewage bacteria beds for different years with the corresponding mean bed temperatures during the periods January–March.

corresponding depression of the catch. Also the seasonal incidence of *A. fenestralis* between May and October 1956 and the sparsity during the following winter period could be attributed largely to the degree of suitability of conditions; however, some of the sharp fluctuations quite disproportionate to suitability changes suggest that other factors affect the aerial density. One such factor is probably a rapidly fluctuating population within the bed as has been demonstrated for other sewage-bed flies by Lloyd (1941, 1943) and later for *A. fenestralis* by Hawkes (1952a). Assuming a thermal constant of 814 day degrees for the development of *A. fenestralis* (Khalsa, 1948) the theoretical recurrence of peak populations was calculated. Three parallel successions of generations were thus traced throughout the whole period as indicated by the vertical arrows on the graph (Fig. 5). The peaks are

sometimes displaced to the right, being delayed by adverse weather; the flies are able to live 20 days at winter bed temperatures and 10 days at summer temperatures. During long spells of adverse weather the emergence peak did not appear at all, the flies being able to complete their full life cycle within the beds.

The following year (1957), however, showed a different incidence of the fly; the population realized its maximum early in the spring and collapsed in June to remain low throughout the summer period when conditions for egress were more favourable. The contrast between the two years is probably due to differences in the incidence of the fly within the bed. Although this was not assessed for the trapping period, previous work (Hawkes, 1952*a*) has shown that it is largely determined by bed temperatures and the availability of food in the film. For other purposes these two factors were assessed on a similar bed and are shown in Fig. 6. The lower winter temperatures of 1955-1956 arrested the development rate of the fly larvae and correspondingly reduced their grazing activity, resulting in a large accumulation of film. Thus early in the spring of 1956 there was a small fly population with sufficient food for it to increase throughout the spring and summer, producing the high incidence shown in Fig. 5. The following winter (1956-57), however, was unusually mild and the more active grazing population restricted the film accumulation (Fig. 6), and thus in the following spring there was not only a larger fly population but a reduced food supply which limited the population later in the year, causing the depression, evident in Fig. 5. Examinations over the past 10 years, as assessed by canister trapping over the beds, show that in 1952, 1953 and 1957 when the mean bed temperatures for January-March were relatively high (see Fig. 7), a maximum population was reached early in the year (April-May) followed by a low summer density. In the other years, when the bed temperature during the first 3 months was lower, the maximum was not reached until later (June-July).

The extent to which fluctuations in the aerial density of *A. fenestralis* are due to population changes or to variations in activity is difficult to assess from the present results. It is hoped that the results of further work will supply the necessary information to account for the fluctuations in aerial density of the fly found in the present work.

This paper is published by kind permission of Mr M. R. Vincent Daviss, M.I.C.E., Engineer to the Board. The author wishes to acknowledge the continued interest of Dr S. H. Jenkins in this work and the initial guidance of Dr C. G. Johnson and Mr L. R. Taylor of Rothamsted Experimental Station and the criticism of the original draft of this paper by Mr Taylor which resulted in its being restricted to a descriptive account, the causes of the diurnal curves being left out to await further evidence. Thanks are also due to Mrs B. Nickless who assisted in the counting of the flies and in the preparation of the paper.

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Experiments on the value of stupefying and lethal substances in the control of harmful birds

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(Received 25 April 1960)

SUMMARY

Laboratory experiments and field trials in town and country were carried out with four reputedly narcotic substances (pentobarbital sodium, dithiobiuret, tri-orthocresyl phosphate and chloralose) and four poisons (picrotoxin, nicotine, parathion and sodium fluoroacetate) to investigate their value in the control of pigeons, corvids and sparrows.

The alpha isomer of chloralose was the most satisfactory material tested; it normally produces narcosis from 15 to 30 min. after ingestion on wheat or maize and this lasts from 10 to 20 hr. Its potency was assessed using treated grain, gelatine capsules and flour pills, administered with and without food, and also from field data. It was most effective on grain and least effective in gelatine capsules. The flour pill method was the most satisfactory for obtaining an LD₅₀ figure which for alpha-chloralose used against feral pigeons was about 131 mg./kg. live weight. The pure beta isomer of chloralose was ineffective.

Using alpha-chloralose, urban populations of feral pigeons were reduced from 48 to 83% in three to seven treatments. In fifty-two trials in towns 1544 feral pigeons were disabled, only five of the 1409 picked up being found at a greater distance than 500 yards from the baiting point. With five different concentrations between 2.5 and 0.5% by weight in bait, the lower concentrations produced lower mortality without affecting the size of the catch. Bait containing chloralose at 1.5% by weight gave the greatest efficiency with a mortality of less than 50% and this concentration is recommended for practical control operations.

In towns the risk of accidentally affecting legally protected species is negligible, whereas in rural districts it is much higher, at any rate when grain baits are used. The use of maize greatly reduces the risk to small birds which cannot swallow the large grains.

Four series of field trials in Kent and East Anglia in autumn and winter caught only seven wood-pigeons and one stock-dove during a total of 72 baiting days. This disregard for cereals is discussed and is thought to be due to changing 'feeding images'.

In four field trials to catch corvids, using alpha-chloralose on maize, only fourteen

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were narcotized. Two field trials using alpha-chloralose against house-sparrows were unsuccessful, but eighty house-sparrows were captured incidentally during four of the trials on feral pigeons. With the possible exception of nicotine none of the other substances tested showed promise for controlling birds. The use of blue-coloured baits, as a warning to the public, reduced takes by feral pigeons but did not prevent feeding altogether.

The value of alpha-chloralose as a control agent and the hazards it presents to wildlife are discussed; its use probably offers a better means of controlling feral pigeons in towns than any existing method.

INTRODUCTION

Damage by wood-pigeons (*Columba palumbus*), rooks (*Corvus frugilegus*) and jackdaws (*Corvus monedula*) to crops, and by feral pigeons (*Columba livia* var.) and house sparrows (*Passer domesticus*) to stored foods and urban buildings has long been a source of complaint by farmers and others. Although it is difficult to measure such damage (Ridpath & Murton, 1956), its reality can hardly be doubted. Wood-pigeon damage to field peas and brassicae is severe in some years and the fouling caused by feral pigeons is a serious cause of complaint in towns.

Present methods of control are costly and of doubtful efficacy and research into new methods of preventing damage by birds is very necessary (Duncan, Wright & Ridpath, 1960). The Protection of Birds Act 1954, allows the use, under licence, 'of poisoned or stupefying bait' to kill or take wild birds of species regarded as harmful and included in the Second Schedule to the Act, and a limited number of licences were issued to research workers from 1955 onwards to allow research into this potential control method. Hitherto the use of such baits had been illegal, except in wartime.

Narcotic baiting has the theoretical advantage over control by rapid-killing agents, such as the gun or poisons, that if legally protected species are caught they may subsequently be released. In practice, however, this advantage does not always apply, because a lethal dose may sometimes be taken.

This paper describes laboratory experiments and field trials on the efficiency of eight stupefying and/or lethal substances as a means of catching birds that damage crops, stored food or buildings. For the present purpose a stupefying substance will be defined as any substance which, when taken orally, induces a period of general external inactivity. Such substances belong to the class described as narcotics in medical and veterinary literature (Henderson, Henderson & Kenneth, 1957). The effects of stupefying substances vary with the dose administered and the body weight and species of the subject. In many cases, the lethal dose is not greatly in excess of that which will cause narcosis; hence the distinction between a narcotic and an outright poison is a small one. A poison may be defined as a substance which does harm to the animal to which it is administered and can cause death, either immediately (acute poison) or after repeated doses (chronic poison). Several narcotic substances fall into one or other category.

The following aspects of the use of stupefying substances have been investigated. The efficiency and cost of such baiting were measured, and information on the average

dose required to stupefy a bird under field and cage conditions was obtained. The most promising of several potential stupefying agents were selected and tried and the risk they presented to man, livestock, game and other wildlife assessed. The question of cruelty was at all times considered and certain substances were specifically rejected because of a possible element of cruelty in their use.

The earliest organized attempts to narcotize wild birds in Britain were made by Colquhoun (1943, unpublished) at Oxford working for the Agricultural Research Council on wood-pigeons. He carried out cage tests and preliminary field trials with tribromoethanol, which appeared promising. Field trials of this substance from 1942 to 1945, with wood-pigeons, had only limited success (Colquhoun, 1946, unpublished) and its use in the present work was rejected because of its chemical instability and its irritant effect on the oesophagus.

Mosby & Cantner (1956) used Avertin (a mixture consisting of 20 parts of tribromoethanol to 1 part of amyl alcohol) in maize bait to catch wild turkeys in Virginia. Owing to the difficulty of getting turkeys to feed on their bait they only caught five, of which one died and four recovered in from 1 to $3\frac{1}{2}$ hr. Narcosis set in between 3 and 17 min. after eating the bait.

Daude (1942), working in France, was the first to show the value of chloralose as a means of catching corvids and other birds harmful to agriculture. Quick-acting poisons, such as strychnine, had long been used to kill corvids in winter, but the war cut off supplies and chloralose was adopted as a substitute. Chloralose is a condensation product of chloral hydrate with glucose. It exists as alpha and beta isomers, and most workers consider only the alpha isomer to have narcotic properties (Giban, 1950). Pure alpha-chloralose is a white crystalline solid with a melting point of $186-187^{\circ}$ C. and is usually obtained in the form of a fine powder. Hariri (1943) defines chloralose as a cerebro-cortical hypnotic which anaesthetizes the brain but has no effect upon the medulla, which is a visceral centre. Excellent reviews of the use of chloralose in France and its effect on corvids, pigeons, game-birds, ducks and poultry have been published by Giban (1950) and Dixméras & Giban (1950). From 1942 onwards maize treated with chloralose was widely used in winter in the northern half of France to destroy corvids, particularly the large flocks of migrant rooks from East Europe that damage young cereals. Records of catches do not appear to exist but the numbers were considered large (Danzel, 1949). In France the maximum concentration of chloralose has now been fixed by law at the relatively low concentration of 0.5 % by weight of bait, to avoid danger to other species, particularly game-birds.

Borg (1955), in Sweden, has determined average lethal doses for the two isomers of chloralose in cage tests, using hooded crows (*Corvus cornix*), magpies (*Pica pica*), feral pigeons, pheasants (*Phasianus colchicus*) and grey-lag geese (*Anser anser*). He also carried out fourteen field trials to catch crows and magpies, one to catch feral pigeons and two to catch herring gulls (*Larus argentatus*), using grain, carrion and fish baits. He concluded that the use of chloralose was humane and that the risks of secondary or accidental poisoning were small, and that it was an efficient method, provided adequate and suitable pre-baiting was carried out. Apart from its use in pest control, chloralose has also been used to catch Corvidae for ringing experiments (Giban, 1954).

Chloral hydrate, the effects of which are similar to chloralose, has been used (Marlow, 1956) to capture the kangaroo, *Macropus robustus*, for marking purposes. The chloral hydrate was dissolved in water in drinking troughs and kangaroos which drank became narcotized for 4-11 hr. Of twenty-six so captured three failed to recover.

Of all the substances considered, chloralose was thought to be the most promising. The research took the following lines: first, four field trials were done with chloralose to catch rooks, in order to compare results in Britain with those obtained in France. Next, four sets of field trials took place in an attempt to capture wood-pigeons, in winter and spring, in Kent and Lincolnshire. These trials were unsuccessful and further trials were done to discover the effects of chloralose on a close relative of the wood-pigeon, the feral pigeon. This bird lives free in towns yet is very tame and accustomed to feed on a wide variety of food provided by man. The object was to see whether chloralose showed sufficient promise to warrant continued research on the wood-pigeon. The urban trials were successful and the method was further investigated to assess its value in controlling feral pigeons in food stores and other urban environments.

LABORATORY TESTS

Cage tests on candidate stupefying substances and poisons

The reputedly stupefying substances tested were pentobarbital sodium (Nembutal), dithiobiuret and tri-ortho-cresyl phosphate; these were selected because of their use in veterinary work. The poisons chosen were picrotoxin, nicotine, parathion and sodium fluoroacetate. It was also decided to test the effect of the beta isomer of chloralose, which was said by Giban (1950) to have no narcotic properties but was claimed to be an excitant, as well as being toxic.

Method. All the tests were carried out on naïve feral pigeons, trapped near Birkenhead, using four or five birds for each substance with one control bird in each case. Two tests were made with beta-chloralose when seven birds were used, with one control in each test.

Each group of birds was starved overnight so that, when the test substance was administered, conditions would be similar to those in the field, where trials were carried out at dawn during the birds' first feed of the day. When the test substance was in powdered form it was given to the birds in flour and water pills. These were made by adding the powder to about 0.5 g. of sterilized white flour and then adding water and mixing into small round pills. With care it was possible to ensure that all solid material was taken up. This technique follows one commonly used in bio-assay work on rodents (Bentley, 1958). Each bird was dosed by opening its bill and gently pushing the pill down the oesophagus with a pair of forceps. Control birds were given a pill of flour only.

Liquid substances were given in a gelatine capsule, the liquid being injected from a 1 ml. hypodermic syringe. The hole made by the needle was sealed with glue and the capsules given to the birds in the same way as the flour pills. Control birds received an empty capsule.

After dosing, the birds were released in an artificially lit room, fitted with an observa-

tion panel of darkened glass and containing a table and a pair of steps to act as perches. The birds were marked distinctively with coloured ink and were placed in a cage in the watching room on the day before each experiment, in order to accustom them to new surroundings. After release, the birds were watched continuously by at least one, and normally two, observers and their behaviour was recorded at regular intervals.

All the solid substances were tested at dosages of 5, 25, 75, 250, and 750 mg./kg. with the exception of beta-chloralose (32.2, 81.0, 156.4, 247, 392, 622 and 986 mg./kg.) and picrotoxin (3.4, 6.8, 13.5, 27.0 and 53.9 mg./kg.) where some previous information was available about their effect. All liquids were administered to groups of birds of equal weight at doses of 0.02, 0.08, 0.2 and 0.8 ml., except with nicotine where the highest dose was 0.4 ml. If any of the test substances showed promise the wide dose range would give some indication of the optimum dose and then further tests could be made using a more restricted range.

Results. Of the stupefying substances, dithiobiuret and tri-ortho-cresyl phosphate had practically no effect and all birds survived. The two lowest dosages of pentobarbital sodium (5 and 25 mg./kg.) produced little effect. The next two concentrations (75 and 250 mg./kg.) produced deep narcosis but the action was very slow. The highest dosage (750 mg./kg.) had some effect after 13 min. and death occurred in 3 hr. Both dithiobiuret and tri-ortho-cresyl phosphate produced vomiting-like movements in the birds.

With beta-chloralose no effect was observed, and this result agrees with that of Hanriot & Richet (1893) who found that beta-chloralose has no narcotic, toxic or excitant properties. Borg (1955), using an American preparation of the pure beta isomer, concluded that its toxicity was about one-twentieth of that of alpha-chloralose. He obtained symptoms of chloralose poisoning at four dosages lower than our highest dosage (986 mg./kg.) which was itself without effect. We cannot account for this difference.

All the direct poisons produced some effect. With picrotoxin both the lowest and highest dosages (3.4 and 53.9 mg./kg.) had no apparent effect, whereas the middle dosage (13.5 mg./kg.) caused trembling and staggering without leading to death. The remaining two dosages (6.8 and 27.0 mg./kg.) both caused death after violent convulsions.

Nicotine at 95% caused rapid death at all concentrations, even the lowest (0.054 ml./kg.) causing death in less than 2 min. Technical parathion at the lowest dosage (0.0625 ml./kg.) produced death after 22½ min. and the bird was violently convulsed before death. At the other dosages death also occurred, but the convulsions were mild.

Sodium fluoroacetate at 90% produced death at all but the lowest dosage (5 mg./kg.). Death occurred slowly, varying between 2 hr. at 750 mg./kg. and 60 hr. at 25 mg./kg. Three of the birds vomited. Although sodium fluoroacetate is a dangerous poison to use in the field, it has been used effectively to destroy large numbers of rooks in New Zealand (Bull, 1957). It has also been used against the Tasmanian native hen (*Tribonyx mortierii*) with little apparent success (Graham, 1957).

Estimation of the median lethal dose for alpha-chloralose to feral pigeons

There is little information available regarding the toxicity of alpha-chloralose to birds, except for the work done by Giban (1950) and Borg (1955). To compare chloralose with other toxic substances, an LD 50 determination was carried out on feral pigeons. Groups of birds were given log-dosages over a range at which all birds survived at the lowest dose and all died at the highest.

Method. Nine groups, each consisting of seven naïve feral pigeons, were used in the test, with one control bird for each group. Each of the nine groups was given alpha-chloralose at dosages of 30.0, 42.4, 60.0, 84.9, 120.1, 170.0, 241.0, 340.0 and 481.0 mg./kg. respectively and the birds were starved overnight before dosing. The chloralose was administered in flour pills as described above, and the birds were kept under observation in cages. Time of dosing was recorded for each bird and all had coloured identification leg rings. Records were kept of the times at which each bird reached the various

Table 1. *Toxicity of alpha-chloralose to feral pigeons*

The figures in parentheses give the average times taken to reach each stage of narcosis.

Group	Dosage (mg./kg.)	No. of birds reaching stage I	No. of birds reaching stage II	No. of birds reaching stage III	No. of birds reaching stage IV	No. of birds dying
A	30.0	7 (1 hr. 6 min.)	6 (1 hr. 8 min.)	3 (1 hr. 26 min.)	—	—
B	42.4	7 (43 min.)	7 (53 min.)	2 (1 hr. 8 min.)	—	—
C	60.0	7 (1 hr. 13 min.)	7 (1 hr. 35 min.)	6 (2 hr. 6 min.)	—	—
D	84.9	7 (30 min.)	7 (48 min.)	7 (1 hr. 20 min.)	1 (4 hr. 30 min.)	1 (5 hr.)
E	120.1	7 (32 min.)	7 (35 min.)	7 (1 hr. 32 min.)	5 (3 hr. 49 min.)	5 (4 hr. 41 min.)
F	170.0	7 (30 min.)	7 (40 min.)	7 (1 hr. 32 min.)	6 (3 hr. 33 min.)	6 (3 hr. 43 min.)
G	241.0	7 (26 min.)	7 (31 min.)	7 (1 hr. 44 min.)	6 (2 hr. 51 min.)	5 (2 hr. 57 min.)
H	340.0	7 (43 min.)	7 (1 hr. 3 min.)	7 (2 hr. 4 min.)	6 (2 hr. 20 min.)	6 (2 hr. 32 min.)
I	481.0	7 (17 min.)	7 (24 min.)	7 (31 min.)	7 (1 hr. 17 min.)	7 (1 hr. 33 min.)

stages of narcosis, the time of death and, where death did not occur, the times of the start of recovery and full recovery. The four stages of narcosis corresponded with those described by Giban (1950) and were as follows:

Stage I: cessation of activity—bird fails to perch properly and staggers in a 'drunken' manner if disturbed. Bird cannot be caught easily. Eyes open.

Stage II: bird stands still in hunched position with eyes closed or flickering. Does not move if approached quietly or at slight noise or movement, but does move if touched. Can be caught with care.

Stage III: bird lies down with head drooping and eyes closed. Remains still except for occasional periods of mild convulsions with wings and tail flapping. Moves when touched or handled. Can be picked up easily.

Stage IV: as stage III but with no convulsions or movements. No reaction when touched. Bird appears to be in deep sleep. This stage usually leads to death.

It was hoped that these observations would give some information about the time of onset and length of narcosis at varying concentrations of chloralose.

Results. The results of the test are summarized in Table 1. It will be seen that at the three lowest dosages, groups A, B and C, no birds died. In group D one bird died,

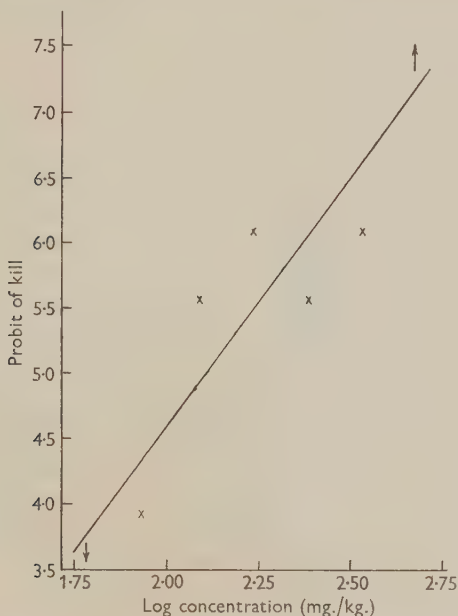


Fig. 1. Relationship between dosage of alpha-chloralose and probit of kill of feral pigeons showing probit regression line.

in groups E and G five birds died, in groups F and H six birds died, and in group I all seven birds died. When the data are analysed in terms of log-dosage and probit mortality (Finney, 1952) an LD₅₀ figure with a maximum likelihood estimate of 130.8 mg./kg. ($\log \text{LD}_{50} = 2.1165$) is obtained (Fig. 1), with a fiducial probability of 95 % that the true LD₅₀ lies between 94.3 mg./kg. and 173.0 mg./kg. The slope of the regression line is 3.8.

The times taken to reach the various stages of narcosis did not follow a very clear pattern. There was considerable variation, not only from group to group as can be seen from Table 1, but also among the individuals within each group. The stages in narcosis

are somewhat arbitrary and it was not always easy to determine whether a bird had reached a certain stage or not: the most difficult to judge were stage I, which was not always evident unless the bird was disturbed, and stage IV which was often hard to distinguish from stage III and was usually a relatively short period preceding death. Birds seldom recovered after having reached this stage. The times for full recovery also varied considerably—the shortest being 3 hr. and the longest 31 hr.

Taking the results as a whole, it seems that a higher concentration of chloralose does produce narcosis more quickly, and that the birds pass through the various stages more rapidly, although this was partly obscured by the effects of individual variation in the small sample of pigeons used for each dosage. A sample size of fifteen to twenty birds at each dosage would probably have given better results. It is clear, however, that the lower concentrations do not produce such a deep narcosis as the higher ones.

Comparison of four methods of dosing feral pigeons with alpha-chloralose

A preliminary LD₅₀ determination had been made with gelatine capsules instead of flour pills and it was clear that the speed of action of alpha-chloralose, when administered in this way, was slower than when given in a flour pill. A test was therefore made to compare the flour pill and gelatine capsule techniques, and also the normal field trial conditions (treated grain) and gelatine capsules in the presence of grain.

Method. Sixteen naïve feral pigeons were divided into four groups and each group was dosed with alpha-chloralose at 250 mg./kg. Group A had flour pills; group B, treated grain; group C, gelatine capsules plus grain; and group D, gelatine capsules. Groups A and D were dosed with chloralose in pills or capsules in the manner described above. Group B was dosed with treated whole wheat as in the field experiments described below, but at a dosage such that forty-seven grains held the equivalent of 250 mg./kg. Forty-seven was the average number of grains in the gizzards of birds killed by alpha-chloralose in the field trials. The birds used in group B were all of the same weight (360 g.) so that by estimating the number of grains in a kilogramme of wheat it was possible to add the correct quantity of chloralose for every forty-seven grains to hold 90 mg. (equivalent to 250 mg./kg. live weight). This dose was poured into the crop of each bird through a glass funnel. The birds of group C were dosed first with gelatine capsules and then with forty-seven untreated grains, administered in the same way.

The times of dosing were recorded and the stages in narcosis noted as in the LD₅₀ test above.

Results. The results of the experiment are summarized in Fig. 2. It will be seen that all four birds died in both groups A and B. One bird died in group C and no birds in group D.

It is clear that with capsules the action of the chloralose is not only much slower but is also less toxic, as no birds died, whereas they all died when dosed with flour pills. Dosing with capsules and grain together showed an increased effect over dosing with plain capsules, although the action was far less potent than with flour pills. The presence of grain, however, appeared to quicken the action as the birds showed some

effect after $\frac{1}{2}$ hr. and had reached stage III in less than 2 hr. (compared with $2\frac{1}{2}$ and $4\frac{1}{2}$ hr. with plain capsules); but only one bird died and the remainder took about 48 hr. to recover.

The effect with treated grain needs further explanation. From the figures it appears

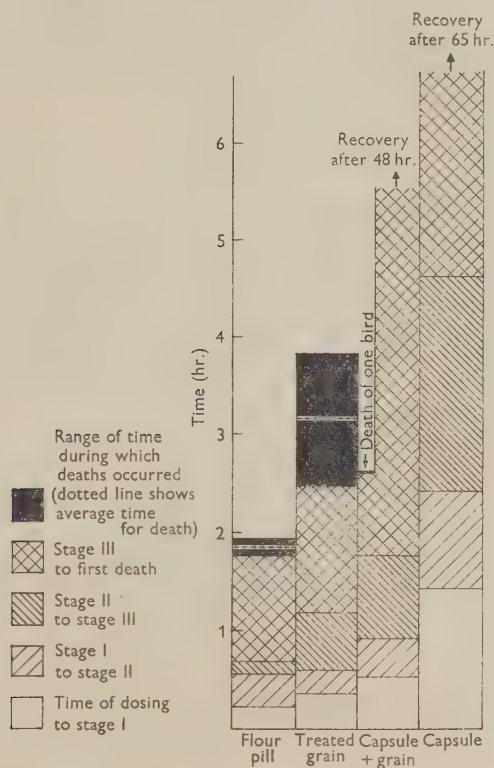


Fig. 2. Comparison of four methods of dosing feral pigeons with alpha-chloralose. Diagram showing the average time to reach the stages of narcosis (stage IV omitted).

to be slower than with flour pills, though eventually leading to the death of all four birds; however, on opening the birds after death, it was found that in each case between twenty and thirty grains had been retained in the crop and could have had little effect. The birds, therefore, received an effective dose of approximately 112 mg./kg. and this comparatively small dose was sufficient to cause death in all cases.

The bird which died in group C was also opened and was found to have twenty-one grains still in the crop—there was no trace of the gelatine capsule in either crop or gizzard. The four birds in group A were examined after death and no traces of the flour pills were found.

To establish that the delays in onset of narcosis, when dosing with capsules, were not caused by the time taken for the capsules to dissolve, tests were made to determine how long the capsules took to dissolve in water at 41° C., which is the approximate blood temperature of a bird (Grassé, 1950). It was found that for the twelve capsules tested, the average time for the capsule wall to break was 52 sec. and the average time for the capsules to dissolve completely was 9 min. This test, however, does not take into account the grinding action or the different medium of the gizzard which would be expected to hasten the break-up of the capsule.

RURAL TRIALS

Corvids

Method. Two sites were chosen in Surrey where rooks and jackdaws were known to feed. One site consisted of a ploughed field surrounded by pasture and woodland; the other was an isolated sewage farm surrounded by arable and pasture. The sites were pre-baited for 3–7 days with whole maize, the residues of which were removed the evening before the operation. To produce a bait containing 0.5 % by weight, a solution of a mixture of alpha- and beta-chloralose was poured on to the base which was then stirred and allowed to dry. The solution was used hot because chloralose is more soluble in hot than in cold water.

2 kg. of treated maize were laid 35 min. before dawn, in twenty patches, over a

Table 2. *Narcotic action of alpha-chloralose on jackdaws and rooks*

(All tests with maize at dawn.)

Place	...	East Horsley, Surrey		Old Woking Sewage Farm	
Date	...	15 Feb. 1955	16 Feb. 1955	16 Dec. 1955	19 Jan. 1956
% chloralose by weight		0.5	0.5	0.5	0.5
Days pre-baiting		3	4	7	6
No. present		60	60	80	80
Minimum number fed		30	9	40	20
Number disabled:					
Died		2 (jackdaws)	0	1	0
Stupefied		7 (2 rooks; 5 jackdaws)	0	1	2
Cause of death uncertain		0	1	0	0
Total		9 (2 rooks; 7 jackdaws)	1 (jackdaw)	2 (rooks)	2 (rooks)
Min. between 1st feeding and 1st symptoms of narcosis		15	—	30	10
Average duration of narcosis (approx. hr.)		5	—	12	6
Max. distance affected birds found (yd.)		200	100	40	150
Man-hours on site		4	4	2	2
Duration of feeding (approx. min.)		7	4	43	50
Protected species:					
Picked up		0	0	2 (chaffinches)	0
Seen to feed		0	0	0	0

length of 25 yards. The area was kept under observation from a concealed position about 200 yards away.

Results. Results of four tests are given in Table 2. A total of six rooks and eight jackdaws were disabled, of which two jackdaws and one rook died and the rest recovered, except for one jackdaw taken by a dog. Two jackdaws, caught on 15 February 1955, were painted with quick-drying yellow cellulose paint on the nape of the neck and were thus identified when they fed on narcotic bait the following morning. All the birds that regained the power of flight were ringed and released. One jackdaw caught on 15 February 1955 was found freshly dead in the same area on 15 April 1957, indicating that recovery from the narcotic was complete.

Two chaffinches (*Fringilla coelebs*) were found stupefied, but recovered and were ringed and released.

The effect of chloralose on the corvids was firstly to disturb their equilibrium after 10–20 min., and then to produce complete torpor, which caused those perched in trees to fall to the ground. One rook on the ground was found half-way down a rat hole only 30 min. after becoming affected. Narcosis lasted from 5 to 12 hr. It can be seen from Table 2 that only a small proportion of those which fed were caught. Searching in woodland and even on pasture is laborious and may be difficult, and this is reflected in the figures for the man-hours spent on the site to pick up only a small number of birds.

Wood-pigeons

Bait was prepared as above except that industrial alcohol was used as a solvent for the chloralose in later tests, as chloralose is more soluble in alcohol than in water.

1955. In March 1955, a preliminary baiting trial of 10 days was done near Tilmanstone in Kent, with maize and field peas treated with 2.5 % chloralose (a mixture of the alpha and a small quantity of the beta isomer) by weight. Small piles of bait, about 6 in. in diameter, were laid in two lines of 100 yards each across a clover ley on which a flock of 800 wood-pigeons was feeding. The area was kept under observation from a vehicle, at a distance of about 200 yards, throughout the hours of daylight. The birds were not seen to feed on the bait, nor were any found narcotized. A second trial lasting 5 days at the same place, in November 1955, also failed to catch any pigeons.

1956. Baiting trials were carried out with maize, buckwheat, maple peas and wheat, all of which pigeons are known to eat. They took place between 19 January and 19 April 1956 in two areas of Lincolnshire (one in Lindsey and one in Holland) where crops such as peas and brassicae suffer severe damage from large flocks of wood-pigeons in spring and winter.

Methods. The baits were laid on fields where pigeons were seen to be feeding and were kept under observation from a vehicle 20–100 yards away, from dawn to dusk for periods of 4–14 days, as shown in Table 3. Eight squares of 10 m. side were laid consisting of one of each of the four baits treated with 2.5 % pure alpha-chloralose, and one untreated control of each. Distance, irregularities of the ground and vegetation sometimes made it difficult to observe feeding by pigeons, and experiments were

done at the outset in Lincolnshire (Lindsey) with four parallel lines of bait, 2 ft. apart, each comprising twenty-six piles of twenty grains each. These were watched and also counted at dawn and dusk. However, the grains disappeared even in the absence of pigeons and it was concluded that these losses were due to voles or field mice. This method was abandoned and we were forced to rely upon watching the baits.

Results. Wood-pigeons were normally present on the fields where the squares of bait had been laid out. The number varied from twenty-five to over 1000 but normally there were between 100 and 500. A period of very cold weather in February and early March, with snow cover and frequent hard frost, caused large feeding concentrations on the fields of attractive crops (brassicæ) where squares had been laid, thus giving pigeons the maximum opportunity of seeing the bait and eating it. A large number of pigeons fed in the area, and torn leaves of brassicæ and faeces on the bait squares proved their proximity to the bait. In spite of this few birds fed on the baits and the only pigeons caught (seven wood-pigeons and one stock-dove) were taken in the relatively mild weather of late March and early April (Table 3). Observations over the entire period of the trials suggested that pigeons fed mainly on green foods between

Table 3. *Narcotic action of alpha-chloralose on wood-pigeons showing the number of days during which baits were under observation, and catches*

	Lincolnshire (Lindsey)		Lincolnshire (Holland)		Total days	Total caught	Protected spp. caught
	Days	No. caught	Days	No. caught			
January 1956	14	0	12	0	26	0	0
February 1956	4	0	0	—	4	0	0
March 1956	6	0	12	6 wood-pigeons	18	6 wood-pigeons	3 pheasants, 3 common gulls
April 1956	9	1 wood-pigeon, 1 stock-dove	0	—	9	1 wood-pigeon, 1 stock-dove	3 skylarks
Total	33	1 wood-pigeon, 1 stock-dove	24	6 wood-pigeons	57	7 wood-pigeons (2 died), 1 stock-dove	3 pheasants (1 died), 3 skylarks (1 died), 3 common gulls (2 died)

January and mid-March, but that when these foods ceased to be available, in late March and April in Lincolnshire, they turned to a more varied diet including carbohydrates, such as small potato debris left on tilled fields and newly sown corn.

Table 3 gives details of the nine birds of protected species which were caught. Besides these, flocks of eleven and forty greenfinches (*Chloris chloris*) with small numbers of chaffinches, were seen to feed on the bait in Lincs. (Lindsey). The greenfinch feeds in open fields on seeds and grain, and narcotic baiting might be a danger to this species unless a large-grain bait such as maize, which it cannot swallow, is used.

URBAN TRIALS

I. *Feral pigeons*

Although the feral pigeon does much less harm to agriculture than the wood-pigeon it is nevertheless harmful to man in several ways (Anon. 1958). It feeds upon and spoils stored food, especially grain in warehouses and in food-processing plants. Food is fouled by the faeces of birds feeding and roosting within the buildings, and so are structures and tackle in warehouses. Employees dislike handling ropes, handles and other apparatus which are soiled with faeces. Accumulations of dried faeces tend to attract insects, such as beetles, moths and flies. The beetles *Dermestes lardarius* and *Ptinus tectus*, both of which are stored-product pests, are commonly found in the nests of feral pigeons (Woodroffe, 1953). Holes may be pecked in bags containing grain and much expense caused through the subsequent spillage.

The possible spread of ornithosis (a virus respiratory disease of non-psittacine birds and man, resembling mild psittacosis) by feral pigeons may present a public health problem. Meyer (1959), in an authoritative review, mentions evidence of transmission of ornithosis to man by feral pigeons in fifteen countries, with a total of 470 cases. Lepine & Sautter (1951) showed that 65 % of the feral pigeons in Paris gave a positive reaction to a complement-fixation test for ornithosis, while 6.25 % of 368 cases of human pulmonary infections in Paris in 1950-51 were diagnosed as ornithosis. However, medical opinion is still divided both on the importance of the feral pigeon as a vector and on the gravity of this disease; nevertheless, the undoubted nearness to man of domestic and feral pigeons makes them potential sources of human ornithosis.

Halls, churches and other public buildings in towns offer suitable nesting and roosting places to feral pigeons and are often seriously fouled by them. The cost of cleaning faeces from such buildings is high, and the problem is of concern to many local authorities. The only existing means of control is the use of cage traps which catch relatively small numbers and are costly in man-hours.

Purely agricultural damage to field peas by feral pigeons can be locally important, and has been reported from Essex and the Wirral area of Cheshire.

Trials using varying concentrations of alpha-chloralose in bait

Method. Fifty trials were done between September 1955 and November 1958, in towns, comprising five sets, of ten trials each, using concentrations of pure alpha-chloralose between 0.5 and 2.5 % by weight in bait. One test was also done at 5.0 % and one at 3.0 %.

Sites for trials were chosen largely from among those about which complaints had been received. Two main conditions had to be fulfilled before a site was considered suitable—there had to be a population of over twenty feral pigeons; and suitable flat baiting points had to be present, at places sufficiently secluded to avoid interference from the public. As far as possible sites were chosen within a 40-mile radius of London, but twelve trials were carried out at Birkenhead, two in East Anglia and one at Birmingham. Twenty took place in food stores or places of food manufacture, one in

a hospital, five in a public garden where plants were being badly damaged and the rest near or on buildings where pigeon excreta had accumulated. Estimates of the numbers of birds present at a site were usually obtained by counting, but useful information was sometimes obtained from employees, such as cleaners and storemen, whose daily work gave them the opportunity to see the local population. Two or three points were chosen for baiting—where possible from the places (such as loading bays, near canteens or near park seats) where pigeons were known to feed habitually on scraps or spillage. Where such natural feeding points did not exist, a spot was chosen and feeding encouraged by a longer period of pre-baiting. On occasions, in the London Docks, spillage was available and constituted a permanent source of food which was made use of effectively as a pre-bait. Otherwise, pre-baiting with 2–6 kg. of grain topped up every 2 days was usually carried out for 7–16 days, depending on the site; periods of 16–28 days were used where a natural feeding point did not exist; periods of less than 7 days were used when one operation followed another closely.

Surplus pre-baiting was used in preference to token baiting (Chitty & Southern, 1954), since pigeons in towns usually have alternative sources of food. During the pre-baiting the number of feeding points was reduced to one, or sometimes two, on the basis of the highest takes. The grain was laid in strips 3 ft. wide and roughly 30 ft. long at a rate of about 100 g./sq.ft. All pre-bait was removed the evening before narcotic baiting.

Grain baits were prepared at first as described for corvids and wood-pigeons, but later the quicker and more convenient technique of applying a sticker and adding dry powdered chloralose was adopted. Colourless technical white oil at 5–8 ml./kg. was mixed thoroughly with grain bait. Chloralose was added slowly and the grain again stirred thoroughly.

In the first test the pigeons preferred wheat to maize, field peas or bread, so wheat was used in all subsequent tests to keep as many factors constant as possible. It is often preferable to use maize as a bait, however, because its grain size is too large for it to be eaten by small protected species (Guillaume, 1951). It may have the further advantage of preventing sparrows taking the bait.

All narcotic trials were done at dawn because (i) the birds had not fed for several hours and might be expected to feed more readily; (ii) the crops and gizzards of the birds would be empty and this would eliminate some variables and also allow the chloralose to take effect more quickly; (iii) the possibility of disturbance of the birds by the public is least in the early morning. Bait was laid in the manner described for pre-bait, half an hour before dawn, and was usually removed before 09.00 hr. It was kept under observation from a concealed position and searching for narcotized birds began 45 min. after the last bird had fed or the first bird had shown symptoms of narcosis, whichever was the earlier.

Results. Results of these trials are given in Table 4. Concentrations of 3 and 5 % chloralose by weight in bait were mainly lethal. At 5 % some of the chloralose fell off the grain on the site (this did not appear to happen at lower concentrations).

The average period between first feeding and the first symptoms of narcosis, in forty-five trials where this could be observed, was 28 min. (range 10–120 min.) and

Table 4. *Feral pigeons—urban trials with chloralose*

(All tests with wheat at dawn.)

Trial	% chloralose by weight	Max. fed at one time	No. disabled				Min. between 1st feeding and 1st symptoms of narcosis	Max. distance affected birds found (yd.)	Duration of feeding (min.)
			Died	Stupefied	Cause of death uncertain	Total			
1	5	7	3	0	4	7	10	15	Not known
2	3	30	26	0	3	29	25	75	9
3	2.5	20	11	9	0	20	13	15	8
4	2.5	6	5	1	0	6	25	15	7
5	2.5	20	12	0	1	13	15	200	20
6	2.5	120	4	3	0	7	16	200	16
7	2.5	32	0	0	32	32	120	2 miles	4
8	2.5	70	26	13	3	42	20	200	40
9	2.5	70	40	0	0	40	26	500	15
10	2.5	49	23	1	19	43	23	200	20
11	2.5	13	7	0	0	7	10	200	4
12	2.5	0	0	0	0	0	—	—	—
13	2.0	12	2	3	0	5	20	100	7
14	2.0	12	3	2	0	5	50	50	25
15	2.0	23	5	4	0	9	17	75	7
16	2.0	0	0	0	0	0	—	—	—
17	2.0	0	0	0	0	0	—	—	—
18	2.0	70	17	35	0	52	17	20	4
19	2.0	18	9	16	0	25	20	20	1
20	2.0	14	3	15	0	18	27	200	5
21	2.0	35	11	21	0	32	19	200	2
22	2.0	235	108	88	0	196	15	400	45 sec.
23	1.5	30	10	2	0	12	40	500	9
24	1.5	25	2	0	20	22	40	2 miles	25
25	1.5	Not known	0	0	2	2	Not known	—	Not known
26	1.5	70	42	20	0	62	15	200	10
27	1.5	5	0	0	1	1	30	10	2
28	1.5	80	20	80	0	100	15	200	26
29	1.5	100	1	11	0	12	20	300	26
30	1.5	40	5	0	0	5	25	200	23
31	1.5	40	11	30	0	41	20	200	11
32	1.5	20	5	13	0	18	30	800	12
33	1.0	54	8	14	4	26	27	5 miles	22
34	1.0	10	3	4	1	8	30	500	58
35	1.0	19	3	5	6	14	38	500	38
36	1.0	23	10	7	0	17	18	2 miles	19
37	1.0	26	3	22	0	25	Not known	100	8
38	1.0	70	9	1	10	20	18	500	24
39	1.0	31	5	1	2	8	18	500	27
40	1.0	2	2	0	0	2	29	25	4
41	1.0	7	1	4	0	5	27	30	9
42	1.0	125	31	65	0	96	15	400	40
43	0.5	35	8	21	0	29	115	75	9
44	0.5	104	31	17	16	64	21	75	13
45	0.5	0	0	0	0	0	—	—	—
46	0.5	Not known	2	1	0	3	Not known	50	Not known
47	0.5	64	5	6	4	15	33	75	13
48	0.5	164	27	20	14	61	18	75	20
49	0.5	38	34	19	1	54	14	50	18
50	0.5	25	5	13	0	18	23	400	11
51	0.5	60	0	6	2	8	58	100	15
52	0.5	60	6	202	0	208	30	100	2

did not vary according to the concentration used. The period of narcosis varied, but usually lay between 10 and 20 hr. (range 2–72 hr.). Sometimes complete recovery was probably delayed due to the passage of more grain into the gizzard from the crop. The stages and nature of narcosis described above could be seen during the urban trials, torpor becoming complete gradually, through stages of vigorous wing and tail flapping, and even disorientated flight, if disturbed. This flight was characterized by being apparently quite normal in birds which had otherwise lost their powers of equilibrium and could neither stand upright nor judge distances in landing or taking off. Some even flew into walls or skidded along the surface of water. Eventually even the power of flight was lost and complete inertia set in.

All birds found were within 500 yards of the site of the trial, except on five occasions when birds with typical symptoms of chloralose poisoning were returned once from $\frac{1}{2}$ mile and 5 miles, and three times from 2 miles. An average of 9.2 man-hours were spent on the site on the day of the narcotic operation, during fifty-two trials. Experimental procedure, however, demanded more time and labour than would be necessary if this method were to become normal pest control practice, although some time would have to be spent in pre-baiting (usually 10–20 min. every 2 days).

A total of 1544 pigeons was known to have been disabled. Of these, 1409 were picked up and we observed how many recovered or died. The remaining 135 were accidentally killed, mainly by drowning or falling from buildings.

Only eleven birds of protected species were seen to feed—six blackbirds (*Turdus merula*), one robin (*Erithacus rubecula*), one pied wagtail (*Motacilla alba*) two mallards (*Anas platyrhynchos*), and one skylark (*Alauda arvensis*)—of which five were picked up, one blackbird and one mallard actually dying. Protected species were caught at five sites, and four of these were near open grass-covered areas or gardens. The exception was at Brentford Dock, where a blackbird and a robin penetrated to the third floor of a warehouse.

Groups of birds spent only a short time feeding on the bait, the average being 14 min. for the forty-five trials in which it could be measured. Furthermore, pigeons (both groups and individuals) sometimes fed rather intermittently, suddenly flying away from the bait, without apparent cause, after only 1–2 min. feeding and returning only after 10–15 min. It is possible that this was due in some way to the narcotic or the presence of the observer, though the observer was usually well concealed at a distance. Moreover, four observations on untreated wheat between 05.00 and 08.00 hr. from 21 to 30 April 1959 showed that this wary behaviour also occurred in the absence of the narcotic—the total feeding periods being 4, 2, 4 and 1 min. on consecutive dates, and 4 min. on the final morning when narcotic bait was used. This behaviour is more likely to have been caused by environmental stimuli sometimes not obvious to the human observer, such as the presence of cats or other predators.

In Fig. 3 the average mortality for the five sets of ten trials has been plotted against the five concentrations of chloralose and a curve calculated and fitted. Inspection shows that an average mortality of 50% should occur between 1.5 and 2.0% by weight of chloralose in bait, and that below 1.5% more birds should be stupefied than killed. A measure of the efficiency of the operations has been obtained by calculating the

percentage disabled of the total known to feed (maximum number fed at one time) at each concentration and these figures have been included in Fig. 3. Efficiency varies between 40 and 75 % but does not appear to be related to the concentration. In compiling these figures all experiments at the Birkenhead Docks have been omitted, as it is known that the maximum number of birds which fed at each operation there bears no relationship to the total of birds present in the area. This was because after the birds resident at the site had fed, relays of birds attracted from surrounding sites came to feed, so that frequently the total of birds caught was considerably greater than the population resident at the site.

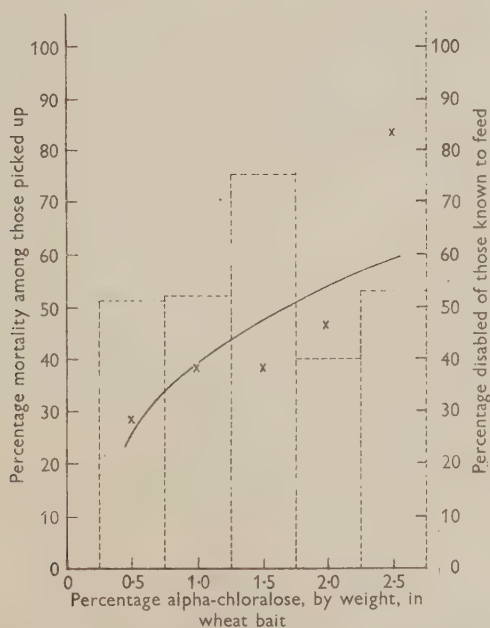


Fig. 3. Relationship between concentration of alpha-chloralose in bait and mortality of feral pigeons in field trials. The dotted histogram shows the percentage of birds disabled of those known to feed at each concentration.

Of the pigeons which had died from chloralose during the tests 255 were dissected in the laboratory and the number of grains contained in crop and gizzard were counted: neither figure bore any relationship to the concentration of chloralose used, but whereas the figures for gizzards were fairly consistent, those for crops were much greater and varied widely (minimum nine, maximum 1679) and probably reflected only the hunger of the individual bird and the time it spent feeding, since the crop is a non-absorptive food storage sac.

At room temperature, 1000 grains of wheat were found to weigh 29.5 g. The average number of grains in the gizzard of 255 birds was found to be 46.9, therefore the

average weight of grain taken into the gizzard by a pigeon was 46.9×0.0295 g. The concentration which would kill 50 % of the birds is 1.7 % by weight of bait as determined from Fig. 3; therefore the average weight of chloralose consumed by the birds which died was $46.9 \times 0.0295 \times 0.017$ g. The average weight of 150 feral pigeons was found to be 362 g., therefore the dosage to kill 50 % of a sample of free-living pigeons during such tests would be $(1000/362) \times 46.9 \times 0.0295 \times 0.017 \times 1000 = 65.0$ mg./kg. of pure alpha-chloralose.

Clearance trials

The success of the bait concentration trials showed that chloralose might be of real value in reducing the feral pigeon population in areas where this bird is a pest. Trials were therefore arranged, with the object of attempting a complete clearance of feral pigeons in some badly infested areas. Three sites were utilized and five clearance operations were done. The first of these was at an R.A.F. Station in Surrey, where two series of trials were held. Here the birds were living in four barrage balloon hangars which were used to store medical equipment. Three of these hangars were close together in a row, whilst the fourth was about 100 yards away on somewhat higher ground. The pigeons caused considerable fouling on the floors of the hangars and the equipment stored in them.

The other two sites were at the Birkenhead Docks. Two series of trials took place at a granary where grain of all sorts was being stored. The birds roosted both inside and on the outside ledges of the store and constituted a great nuisance, especially by fouling the stored grain. Immediately behind the granary was a second store, separated from the first by a strip of water which joined the main docks behind, and in front was an area of waste ground where much of the baiting was done.

The other Birkenhead series was done at a mill, situated about half a mile from the granary and in similar surroundings. Here, again, large quantities of stored grain were being fouled.

Method. The method used was the same as in the bait concentration trials, and the same observations and precautions were taken. Maize or wheat containing between 0.5 and 2.5 % by weight of chloralose was used. The time between each operation of a series varied from 2 days to 1 month; on only one occasion were narcotic operations performed on successive days.

All the operations at Birkenhead were attended by officers of the Liverpool Branch of the R.S.P.C.A. A small boat was used to remove narcotized birds from the adjacent stretches of water.

Results. In the first series of Surrey trials it was estimated that about forty pigeons were present at the start. In seven trials nineteen birds were taken, so its efficiency was about 48 %. The second series took place 15 months later, when it was estimated that approximately 100 birds were present. During the interval between the two series there had obviously been a big build-up of birds—a situation which is always likely to occur. Three operations took place in the second series and a total of fifty birds was taken, an efficiency of about 50 %.

In the first series at the Birkenhead granary the population of feral pigeons was

about 1100 before operations commenced. Seven trials took place and 911 birds were taken—an efficiency of clearance of about 83 %. In fact the clearance may have been more successful than this, for at the start of the second series of trials, 11 months later, only about eighty birds were present. On this occasion eight trials were held and 605 birds taken. It appears that many more were being taken than were resident at the site, because pigeons were attracted to the bait from other parts of the docks.

In the final series, at the mill, the population of birds was approximately seventy-five. Four trials were held and 198 birds taken. Here again birds were being attracted from other parts of the docks. Estimates of the remaining resident population in these last two series suggest that about 50 % clearance was achieved.

The number of birds feeding decreased during each of the series, and the numbers captured dropped in most cases. However, in the second series at the granary, and also the trials at the mill, numbers of feeding birds rose again towards the end, presumably due to their being attracted from the surrounding areas. The fall in numbers would be expected if the population were being reduced all the time, but bait-shyness may have played an important part, as may have the increased occurrence of cats, which seemed to be attracted by the regular presence of feeding birds. Cats were present both at the Surrey trials and at Birkenhead. In the first Surrey series a change from wheat to maize bait was made towards the end of the trials, because the presence of narcotized sparrows was thought to be preventing pigeons from feeding: this change, however, had no effect. No birds were caught during the last four trials of this series as none fed. The bait was ignored and the birds flew away first thing each morning—it was thought that they were feeding at a nearby zoo.

Use of coloured bait

Alpha-chloralose is a stable compound and would constitute a hazard to any grain-eating animal if left on the site of a clearance operation. Although this is equally true during the period of clearance, the presence of the operator should ensure the bait is not eaten by domestic animals. In cases where this does not apply or where it is difficult to sweep up all the bait, it would be an advantage to dye the grain and thus warn anyone finding it.

Work by Kalmbach (1943) and Kalmbach & Welch (1946) has suggested that certain colours are repellent to birds (a disadvantage from the present viewpoint), but that blue is the least repellent. Mansfeld & Bösenberg (1955) showed that house-sparrows would accept blue-dyed wheat under field conditions and that it was slightly preferred to green. English (1953) found that gentian violet failed to prevent bullfinches (*Pyrrhula pyrrhula*) from eating fruit buds in field trials. We therefore experimented with blue-dyed wheat as a warning colour, to discover whether feral pigeons would take it, and how the take compared with that of undyed wheat. The experiment was done just after dawn on 20 September 1957 on a terrace on the south bank of the Thames at Richmond. Eight squares, of 30 cm. side, of wheat coloured with 0.1 % chlorazol blue (total 960 g.) were laid out with eight similar undyed control squares in a chequer board pattern. A maximum of seventy pigeons fed and the uncoloured controls were eaten in 12 min.; then the birds were disturbed and the remaining blue

wheat weighed. As against the 100% take of controls 29% by weight of the blue bait had been taken. It is likely that more would have been eaten had the seventy pigeons been allowed to continue to feed on it.

A further experiment with wheat dyed with 0.1% chlorazol blue, without the choice of uncoloured wheat, was carried out in May 1959 at St Albans. A small population of feral pigeons was pre-baited for a week with wheat and offered blue-dyed bait on the eighth day at 04.00 hr. G.M.T. when fourteen pigeons fed on the blue bait for 5 min.

II. *House-sparrows*

Sparrow damage to crops, food stores and buildings does not rank in importance with that done by wood-pigeons or feral pigeons, and only two experiments were done with the sole object of catching house-sparrows.

The first of these was at London Airport to remove sparrows, the nests of which were liable to cause fires in the hangars. Counts put the population at a minimum of fifty though the real total was likely to have been a good deal higher. Thirty-seven baiting points were chosen in seven small outside hangars and the large main building, and pre-baiting with 1½ cwt. of wheat took place between 31 July and 15 August 1956.

Table 5. *Narcotic action of alpha-chloralose on house-sparrows*

(All tests with wheat at dawn.)

Place	...	London Airport	Kingston Memorial Garden	Kingston river front	Royal Albert Dock	Joyce Green Hospital, Kent	Totals
Date, 1956	...	16 Aug.	30 Aug.	31 Aug.	21 Oct.	5 Dec.	
% chloralose by weight		5	1.5	1.5	1.5	1.5	—
Days pre-baiting		16	8	14	Permanent	14	—
No. present		50+	Not known	Not known	Not known	Not known	—
Minimum number fed		8	19	45	5	30	107
Number disabled:							
Died		1	3	32	4	1	41
Stupefied		0	16	11	1	11	39
Cause of death uncertain		3	0	0	0	1	4
Total		4	19	43	5	13	84
Min. between 1st feeding and 1st symptoms of narcosis		10	20	20	Not known	7	—
Average duration of narcosis (approx. hr.)		—	13+	13+	4	7	—
Max. distance affected birds found (yd.)		15	30	40	20	50	—
Man-hours on site		24	5	13½	20	4	—
Duration of feeding (approx. min.)		Not known	10	15	Not known	50	—

Although instructions were given that this bait should not be removed, some was swept up. Poisoned bait was laid at all points between 03.30 and 05.00 hr. G.M.T. on 16 August, and was removed at 09.30. Although sparrows were active in all hangars none fed on the bait. In the main building at least eight house-sparrows fed and four

were disabled, of which one died. The existence of scraps, sandwich and cake crumbs, combined with interference with baits were the main reasons for the low catch.

The second trial was done at Goudhurst, Kent, in May 1957 when wheat treated with 2% chloralose was laid, after 14 days' pre-baiting, in a hop-garden where a flock of 200 house-sparrows was present. The bait was in position from 04.30 to 15.30 hr. G.M.T., but no sparrows were caught although one blackbird and one robin were seen to feed and were partially affected.

Although the two trials against house-sparrows were not successful, sparrows have been caught incidentally in the feral pigeon trials described above. Details of the numbers caught in four of the feral pigeon trials are given in Table 5, together with the results of the London Airport trial. Out of a total of 107 sparrows which fed, eighty-four were disabled of which forty-one died and thirty-nine recovered (information was lacking for a further four). In several of the feral pigeon trials, although many sparrows were seen to feed, none was found narcotized afterwards. The reason for this observation is not known, but may be connected with the small number of grains a sparrow can eat at one time. The average number of grains in the gizzards of thirty-eight sparrows found dead during trials was five and did not vary with the concentration of chloralose used.

DISCUSSION

In view of the results obtained in the cage tests most of the candidate substances were rejected as agents for the control of harmful birds, because they were ineffective, inhumane or slow in action. Although in the control of some pests, slowness in action is not considered a disadvantage, with birds it is essential that the action should be as rapid as possible to reduce the risk of interference by the public and to prevent poisoned bodies from being scattered widely over populated areas.

The two most effective substances tested were parathion and nicotine. Parathion was finally rejected because it was considered unsafe to use high dosage rates in the field and at low concentrations it produced convulsions in the birds. Nicotine showed more promise, its very rapid action causing death, even at low dosages. It seems probable that, using nicotine, birds would be killed so quickly that they would fall at, or very near to, the baiting point. This would save much time normally spent searching for affected birds. It was not possible, however, to test nicotine in the field, and it is not known whether the death of the first birds to feed would prevent other birds from feeding.

It has been shown that alpha-chloralose is most effective, weight for weight, when spread on the surface of grains, as in the field trials. Gelatine capsules delayed the action of chloralose and reduced its effect, as compared with administration on grains or by flour pills. Borg (1955), using the gelatine capsule method, obtained an LD 50 of approximately 200 mg./kg. for feral pigeons, which is appreciably higher than our figure of 131 mg./kg. which was obtained with flour pills. Using field data and grain counts an even lower LD 50 of 65.0 mg./kg. was estimated for chloralose on grain and this confirms the experimental result that alpha-chloralose is most potent when taken on grain.

The considerable difference in action resulting from dosing with flour pills, gelatine capsules or grain is unexplained. In the tests the birds were dosed after being starved overnight and it seems that the presence of some food in the gizzard assists the action of the chloralose, perhaps by stimulating the digestive processes. In rats it is known that the degree of emptiness of the stomach may affect the toxicity of some substances (Bentley, 1958). The flour pill was equivalent to dosing the birds together with some food. As forty-seven grains of wheat, the average gizzard content, present a larger surface area than a flour pill, dose for dose, it is possible that the entire dose of narcotic is quite rapidly transferred from the grains to the intestine, where it is absorbed; whereas in the case of the flour pill and gelatine capsule diffusion and mixing has to take place in the gizzard before absorption can occur. Dosing with gelatine capsules plus untreated grain did not increase the potency of chloralose to the level of that of the flour pill or grain method of presentation (Fig. 2), therefore it would seem that it is not simply the presence of food or bulk as such, but at least equally, the surface area of presentation which is the determining factor in the potency of a given dose.

The average efficiency of urban field tests, as measured by the percentage disabled of the maximum seen to feed at one time, varied between 40 and 75 % (Fig. 3). This method of assessing efficiency is not very accurate and a more useful standard is the percentage of feral pigeons removed from a given site by a series of operations, as described above. It is difficult to estimate the feral pigeon population before and after the operations and errors arise because movements of birds within an area may cause considerable fluctuations in number, but it is the better way of assessing the efficiency of control operations (Ridpath, Thearle & Jones, in preparation).

Control of feral pigeons with alpha-chloralose is an improvement on existing techniques, but a return of pigeons to cleared sites must be expected, not only from surrounding areas, but also because pigeons are frequently lost or set free by their owners. To be permanently effective, therefore, narcotic control would have to be done on an extensive basis and regularly. The urban trials were expensive in labour, but in normal work this would be considerably reduced. The use of narcotics could thus be a more economic means of feral pigeon control than either trapping or shooting, in terms of birds per man-hour.

The speed of onset of narcosis by pure alpha-chloralose did not seem to differ greatly as between feral pigeons and corvids, nor did the mortality at 0.5 %; however, Giban (1950) found that corvids were about five times more sensitive to chloralose than pigeons and three times more sensitive than partridges. Danzel (1949) has suggested that the reduced sensitivity of partridge and pigeon, compared with corvids, is due to the fact that the former possess a non-absorptive crop between bill and gizzard and this somehow reduces the potency of a given dose of narcotic bait, perhaps by slowing down its passage to the gizzard and mixing it with other food. Our data for corvids are insufficient to refute this hypothesis, and since the pigeons in both the urban and laboratory trials had empty crops it is unlikely that any difference in effect would have been apparent. The small feeding concentrations and numbers disabled (Table 2) during the four field trials to catch corvids are probably related to the weather conditions at the time, which were mild in spite of the fact that it was winter. Giban (1947)

states that big catches are only obtained in cold weather, when there has been continuous frost or snow cover for several days.

The problem of the accidental destruction of harmless and legally protected birds and game-birds is of great concern to many interests. The figures of such catches are summarized in Table 6. If these numbers are grouped into two categories, urban and rural trials, we obtain the following comparisons:

Urban. Sixty-two baiting days gave a catch of 2455 harmful birds (feral pigeons): 11 birds of protected species fed on the bait (2 died, 3 were stupefied and 6 were only seen to feed).

Rural. Seventy-six baiting days gave a catch of 22 harmful birds (corvids, wood-pigeons and a stock-dove): 64 birds of protected species fed on the bait (4 died, 7 were stupefied and 53 were only seen to feed).

Table 6. *Numbers of protected birds accidentally caught or seen to feed during narcotic experiments*

	Died	Stupefied	Only seen to feed	Total
Blackbird (<i>Turdus merula</i>)	1	1	5	7
Robin (<i>Erithacus rubecula</i>)	—	—	2	2
Chaffinch (<i>Fringilla coelebs</i>)	—	2	—	2
Pied wagtail (<i>Motacilla alba</i>)	—	—	1	1
Common gull (<i>Larus canus</i>)	2	1	—	3
Greenfinch (<i>Chloris chloris</i>)	—	—	51	51
Mallard (<i>Anas platyrhynchos</i>)	1	1	—	2
Pheasant (<i>Phasianus colchicus</i>)	1	2	—	3
Skylark (<i>Alauda arvensis</i>)	1	3	—	4
Total	6	10	59	75

These figures show clearly that the risk to protected species in towns is negligible, provided operations are not done near gardens or parks. In the countryside, however, the potential hazard to protected species, including game-birds, is greater. More selective baits than grain might overcome this difficulty.

The immediate problem in the use of narcotics to catch wood-pigeons, or indeed any birds in rural areas, is to find a bait that the birds will take.* Between January and mid-March, wheat with a starch equivalent of 71.6 (Woodman, 1944) was ignored by wood-pigeons in favour of drum-head cabbage with a value of 10.3 starch equivalents per 100 lb. There may be an ethological explanation of this fact. Thorpe (1956) quotes observations by Craig (1912) on the drinking behaviour of young doves. The drinking movement itself is innate, but the recognition of water as being the stuff to drink is not, and has to be learnt. Similarly, the wood-pigeon probably learns to feed on new foods partly by trial and error and once conditioning to a particular food has occurred there will be little tendency to change from it until another food has become sufficiently widely available for its 'feeding image' to have been learnt, or re-learnt, or until the first food has disappeared. This, in fact, is what seems to happen. Some

* Recent work has suggested that wood-pigeons will accept a grain bait provided the bait is very thinly scattered over a wide area.

green foods, such as brassicae, diminish in availability, locally, in March and cereals suddenly become more abundant and available as a result of spring sowing in March and April. Colquhoun (1951) published data which suggest that in March and April the amount of clover, weeds, brassicae and tree fruits, taken together, falls as compared with the period December to February. It is interesting to notice that the only catches of wood-pigeons with baits did in fact take place in March and April.

The problem of finding a bait that is both attractive and selective is one that can best be solved by more research on the feeding habits and food preferences of the wood-pigeon throughout the year.

We are very grateful to our colleagues: Mr A. Taylor, for advice and criticism and for the preparation of pure beta-chloralose; Mr R. K. Murton, for his help in the field at the beginning of this work; Mrs M. Vizoso, Messrs H. V. Thompson, B. C. Mollison and Dr E. W. Bentley for advice and comments on the manuscript; to our colleague Mr W. Chapple for his invaluable work at Birkenhead; to Messrs Staggs and Smith and to Kingston Corporation for their extensive help and co-operation; to our colleagues Messrs H. G. Lloyd and K. L. Turns for assistance and Messrs E. N. Wright and D. D. B. Summers for carrying out the trial at Goudhurst, Kent; to the following organizations, who have helped us carry out trials: Disinfestation Ltd., G.P.O., B.O.A.C., R.A.F., Port of London Authority, the local authorities of Richmond, New Malden and Surbiton, the Ford Motor Co. Ltd., the Royal Society for the Prevention of Cruelty to Animals, British Railways and to Messrs Brookes, Hoyland, Nicholas, Sharp, Gardner and Woodward; to M. J. Giban of the Institut National des Recherches Agronomiques, Versailles; and to the following organizations from whom we have had advice and information on narcotics: the Central Veterinary Laboratory of the Ministry of Agriculture, Fisheries and Food, the Royal Veterinary College, I.C.I. Game Services and Plant Protection Ltd.

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Investigations on fungitoxic derivatives of salicylaldehyde

I. The 3-, 5- and 3,5-chlorinated derivatives of salicylanilide and salicyl-*para*-chloroanilide

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(Received 11 May 1960)

SUMMARY

The eradivative and protective activities of the salicyl-, 3-chlorosalicyl-, 5-chlorosalicyl- and 3,5-dichlorosalicyl-anilides and -*para*-chloroanilides, against cucumber powdery mildew (*Erysiphe cichoracearum* DC.) and tomato leaf-mould (*Cladosporium fulvum* Cke.) have been determined by *in vivo* glasshouse tests on pot plants. No compound was more effective than salicylanilide against either disease at a non-phytotoxic concentration.

In addition, the *in vitro* fungitoxic activities of these compounds against spores of tomato leaf-mould have been determined; several were considerably more active than salicylanilide.

INTRODUCTION

The fungitoxic activity of salicylanilide was reported by Fargher, Galloway & Probert (1930). This compound was subsequently subjected to tests against plant diseases (Bewley & Orchard, 1932; Barrowcliff & Sharp, 1932; Callan & Sharp, 1933; Martin & Salmon, 1934) and was developed as an horticultural fungicide.

During recent years salicylanilide has been largely replaced by other fungicides but, although not a highly active fungicide and having protective rather than eradivative activity, it has the advantage of very low toxicity to plants and animals and it leaves deposits which are, unlike those left by some other fungicides, of negligible importance on certain crops, such as glasshouse tomatoes (Beaumont, 1954).

Salicylanilide sprays have been used extensively in glasshouses during the last 20 years as protectants against tomato leaf-mould (*Cladosporium fulvum* Cke.), and have recently been reported (unpublished work) to give a satisfactory protection against this disease when applied as 'low-volume' sprays. Salicylanilide sprays may, however, be unsatisfactory under conditions of severe leaf-mould infection; for example, Wiggell (1958) has reported that a standard salicylanilide spray gave poor control of tomato leaf-mould under severe disease conditions in 1956. Furthermore, salicylanilide sprays are not satisfactory for the control of cucumber powdery mildew (*Erysiphe cichoracearum* DC.) in commercial practice.

The present work was therefore undertaken with a view to determining whether any of the more easily obtainable chlorinated derivatives of salicylanilide were more

effective against these diseases than salicylanilide, and equally safe. In addition, the *in vitro* fungitoxic activities of these compounds have been assessed against spores of *Cladosporium fulvum* Cke.

Recently, Topps & Wain (1957) have found a series of 3- and 5-alkyl-salicylanilides and -salicyl-4-chloroanilides to be, in general, less toxic to six fungi than the parent compound, salicylanilide.

EXPERIMENTAL

Chemicals

The compounds were, except where stated, prepared by the gradual addition of a solution of the appropriate acid chloride (1 mole) in dry benzene to a stirred solution of aniline, or the appropriate chlorinated aniline (2.05 moles), in dry benzene at room temperature. After removal of the amine hydrochloride by filtration, the filtrate was extracted with very dilute sodium bicarbonate solution, dilute hydrochloric acid and, finally, water, and dried over anhydrous magnesium sulphate. The benzene solution was concentrated under reduced pressure and cooled to precipitate the derivative of salicylanilide, which was recrystallized to constant melting-point.

Salicylanilide (m.p. 135° C.). A commercial sample was recrystallized from ethyl alcohol. (Found: C, 73.2; H, 5.05. $C_{13}H_{11}NO_2$ requires C, 73.2; H, 5.2 %.) The literature m.p. is 135° C.

Salicyl-4-chloroanilide (m.p. 167–168° C.). Crystallized from benzene in white platelets. (Found: Cl, 14.2, $C_{13}H_{10}NO_2Cl$ requires Cl, 14.3 %.) Topps & Wain (1957) give m.p. 167–168° C.

3-Chlorosalicylanilide (m.p. 163.5° C.). Crystallized from 70 % ethyl alcohol in long colourless needles. (Found: Cl, 14.6. $C_{13}H_{10}NO_2Cl$ requires Cl, 14.3 %.) Anschütz (1906) gives m.p. 158.5–159.0° C.

3-Chlorosalicyl-4-chloroanilide (m.p. 225–226° C.). Crystallized from benzene or 95 % ethyl alcohol as white micro-crystals. (Found: Cl, 25.5. $C_{13}H_9NO_2Cl_2$ requires Cl, 25.15 %.)

5-Chlorosalicylanilide (m.p. 203–203.5° C.). Prepared by the method of Hirwe, Jadhav & Sukhtankar (1939). A mixture of salicylanilide (21.3 g.) in dry benzene (180 ml.) and sulphuryl chloride (13.5 g.) in dry benzene (40 ml.) was heated under reflux for 5 hr. The mixture was cooled at c. 5° C. overnight, filtered, and the solid product washed with benzene. Yield of crude product, 95 %. Recrystallization from ethanol/benzene (1:1) mixture gave minute white needles. (Found: Cl, 14.5. $C_{13}H_{10}NO_2Cl$ requires Cl, 14.3 %.) Hirwe *et al.* (1939) give m.p. 203–204° C. The substitution of chlorine into the 5-position was confirmed by the yield of 5-chlorosalicylic acid following hydrolysis with boiling 65 % sulphuric acid.

5-Chlorosalicyl-4-chloroanilide (m.p. 215° C.). The attempted preparation of 3,5-dichloro-salicyl-2,4-dichloroanilide (Hirwe *et al.* 1939), by the prolonged action of sulphuryl chloride (0.4 mole) on salicylanilide (0.1 mole) in boiling benzene (200 ml.), gave a product which crystallized from benzene/ethanol (5:1) as minute white needles and gave the following analysis. (Found: Cl, 25.6; N, 5.3. $C_{13}H_7NO_2Cl_4$ requires Cl, 40.4; N, 4.0 %.) This analysis corresponds to a dichlorosalicylanilide ($C_{13}H_9NO_2Cl_2$ requires Cl, 25.15; N, 4.9 %).

Hydrolysis of the material with boiling 65 % sulphuric acid gave 5-chlorosalicylic acid and 4-chloroaniline (mixed m.p. determinations), confirming that the material is 5-chlorosalicyl-4-chloroanilide. Hirwe *et al.* give m.p. 215–216° C.

3,5-Dichlorosalicylanilide (m.p. 135° C.). Crystallized from benzene in small colourless needles. (Found: Cl, 25.5. $C_{13}H_9NO_2Cl_2$ requires Cl, 25.15 %.) Anschütz (1906) gives m.p. 134–135° C.

3,5-Dichlorosalicyl-4-chloroanilide (m.p. 188–189° C.). Crystallized from benzene in colourless micro-prisms. (Found: Cl, 33.6. $C_{13}H_8NO_2Cl_3$ requires Cl, 33.6 %.)

Salicylic acid (m.p. 159° C.) and 5-chlorosalicylic acid (m.p. 172° C.) were obtained in pure form from commercial samples by recrystallization.

3,5-Dichlorosalicylic acid (m.p. 220° C.) was prepared by the chlorination of salicylic acid in hot glacial acetic acid, and was recrystallized from ethyl alcohol.

In vitro fungitoxic activity

A modified test-tube dilution/spore germination technique (American Phytopathological Society, 1947) was used to estimate *in vitro* fungitoxic activity.

Spores were obtained from a 14-day-old culture of *Cladosporium fulvum* Cke. on oatmeal agar by shaking the tube following the addition of distilled water, filtration of the resulting spore suspension through muslin, and the removal by centrifugation of the liquid and two additions of water for washing the spores. The spore mass was then dispersed in distilled water and the concentration of spores adjusted to 500,000/1 ml.

Suspensions of the test compounds were prepared by adding a solution of the compound in ethyl alcohol to a vigorously stirred aqueous solution of methyl ethyl cellulose, a dispersing agent. The solutions or suspensions of the compounds for assay were prepared by diluting this suspension, with adjustment of the concentrations of alcohol and methyl ethyl cellulose to 0.3 and 0.003 % respectively. These amounts of alcohol and suspending agent did not affect spore germination.

1 ml. of the spore suspension was added to a mixture of 2 ml. of the fungicide suspension and 1 ml. of a stimulant solution (containing 0.005 % dextrose, 0.04 % potassium dihydrogen phosphate and 0.02 % magnesium sulphate). Three 0.03 ml. drops of each test suspension were pipetted on to glass slides, and were incubated at 23° C. in Petri dishes contained in a moist chamber.

Spore germination counts were made after 20–24 hr., and the ED₉₅ dosage was determined in each case by the use of logarithmic-probability paper.

In vitro tests against spores of cucumber powdery mildew were not made, owing to the unsatisfactory germination of these spores under *in vitro* conditions.

In vivo fungitoxic activity

The *in vivo* fungitoxic activities of the above compounds, against leaf-mould (*Cladosporium fulvum*) on tomato plants (var. Ailsa Craig) and powdery mildew (*Erysiphe cichoracearum*) on cucumber plants (var. Butchers Disease Resister), were determined by applying these compounds to the plants in aqueous sprays containing 0.04 % of methyl ethyl cellulose as a dispersing and wetting agent. In each case, the test material was ground repeatedly with a little of a 0.5 % aqueous solution of methyl ethyl cellulose, with repeated additions of water and decantation of the supernatant suspensions. The resulting stable suspension was diluted with the required volume of methyl ethyl cellulose solution to give a 0.005 and/or 0.0025 molar suspension for spraying.

For leaf-mould experiments four uniform tomato plants, about 18 in. high, were used for each concentration of a compound both in tests to determine protective action and in tests to determine eradivative action. To assess protective action, three or more spray applications were made at intervals, commencing with apparently

uninfected plants. In the cucumber powdery mildew trials three plants were used for each concentration of a compound in each test for eradivative activity, and for protective activity. Control plants were sprayed with aqueous 0.04 % methyl ethyl cellulose.

During tests for eradivative activity against cucumber powdery mildew very hot, humid conditions were avoided, since such climatic conditions may cause a pronounced reduction in the virulence of the pathogen, making it more susceptible to fungicidal action.

Tomato leaf-mould inoculations were made twice-weekly, by spraying the plants uniformly with a very dilute aqueous suspension of spores of *Cladosporium fulvum* Cke., obtained from a culture on oatmeal agar; the glasshouse having been 'damped-down', and ventilators closed for the night, before spraying. In addition, several tomato plants with severe leaf-mould infection were maintained at some distance from the test plants, to provide natural inoculum. Inoculum for cucumber powdery mildew infection was provided by several severely infected plants within the glasshouse.

All plants used in any one trial were re-randomized in the glasshouse following each spray treatment.

Recording of the results

On each treated plant an estimate of the percentage area of each leaf visibly infected by the fungus, excluding leaves which were small at the time of the last application of the chemical, was made 7 days after spraying in all eradicant tests and, in protectant tests, 25 days after the final spraying of tomato plants and 9 days after the final spraying of cucumber plants. Similar estimates of infection were made on control plants. Tomato leaf-mould and cucumber powdery mildew are capable of forming visible infections 18–21 days and 7 days, respectively, after inoculation.

In presenting these results, compounds have been graded into three categories: I, giving 95–100 % disease control; II, giving 50–95 % control; and III, weakly fungicidal.

RESULTS

Eradicative activity against tomato leaf-mould

None of the compounds examined gave, at a non-phytotoxic concentration, a satisfactory control of a visible infection of leaf-mould. At 0.04 %, both 3,5-dichlorosalicylanilide and 3,5-dichlorosalicyl-4-chloroanilide killed well-established lesions, but not the very young lesions. This eradivative action, however, appeared to result from the killing of the underlying leaf tissues, which were made more susceptible to chemical injury by severe fungal invasion (these two compounds caused scorching of normal tomato foliage at 0.075 %). A similar effect on older lesions was obtained with salicylic acid at 0.08 %, 5-chlorosalicylic acid at 0.03 %, and 3,5-dichlorosalicylic acid at 0.03 %.

The remaining compounds had no appreciable effect on leaf-mould when applied at 0.12 %, at which concentration 3-chlorosalicylanilide was phytotoxic.

Table 1. *In vitro fungitoxic activity against spores of Cladosporium fulvum*

Compound	ED 95 (in parts per million)
Salicylanilide	6.0
Salicyl-4-chloroanilide	4.0
3-Chlorosalicylanilide	> 50.0
3-Chlorosalicyl-4-chloroanilide	> 50.0
5-Chlorosalicylanilide	1.2
5-Chlorosalicyl-4-chloroanilide	0.4
3,5-Dichlorosalicylanilide	2.0
3,5-Dichlorosalicyl-4-chloroanilide	0.6

Table 2. *Protective activity against tomato leaf-mould*

Compound	Concentration in spray (%)	Disease-control index (I = > 95 % control II = 50-95 % control III = < 50 % control)
(a) Spray applications made on 19, 29 June, 9 and 19 July 1956		
Salicylanilide	0.050	II
Salicyl-4-chloroanilide	0.060	II
3-Chlorosalicylanilide	0.060*	I
	0.030	III
3-Chlorosalicyl-4-chloroanilide	0.070	III
5-Chlorosalicylanilide	0.060	III
5-Chlorosalicyl-4-chloroanilide	0.070	III
3,5-Dichlorosalicylanilide	0.070*	I
	0.035*	I
3,5-Dichlorosalicyl-4-chloroanilide	0.080*	II

Mean control infection was 45 % of leaf area

(b) Spray applications made on 16, 24 August, 3 and 12 September 1956

Salicylanilide	0.10	I
3,5-Dichlorosalicylanilide	0.012	II

Mean control infection was 35 % of leaf area

* Caused injury to the foliage.

Table 3. *Eradicative activity against cucumber mildew*

Compound	Concentration in spray (%)	Disease-control index (I = > 95 % control II = 50-95 % control III = < 50 % control)
Salicylanilide	0.10	II
Salicyl-4-chloroanilide	0.12	II
3-Chlorosalicylanilide	0.12*	III
3-Chlorosalicyl-4-chloroanilide	0.13	III
5-Chlorosalicylanilide	0.12	II
5-Chlorosalicyl-4-chloroanilide	0.13	III
3,5-Dichlorosalicylanilide	0.06*	I
	0.03	II
3,5-Dichlorosalicyl-4-chloroanilide	0.07*	II

Mean control infection was 60 % of leaf area

* Caused injury to the foliage.

Table 4. *Protective activity against cucumber mildew*

Dates of spray treatments (1956)	Compound	Concentration in spray (%)	Disease-control index (I = > 95 % control II = 50-95 % control III = < 50 % control)
20, 28 June,	Salicylanilide	0.05	II
10 and	Salicyl-4-chloroanilide	0.06	II
20 July	3-Chlorosalicylanilide	0.06	III
	3-Chlorosalicyl-4-chloroanilide	0.07*	II
	5-Chlorosalicylanilide	0.06	III
	5-Chlorosalicyl-4-chloroanilide	0.07	II
	3,5-Dichlorosalicylanilide	0.07*†	I
	3,5-Dichlorosalicyl-4-chloroanilide	0.08*†	I

Mean control infection was 70 % of leaf area

* The 10 July spraying caused mild leaf-scorch.

† Caused a reduction in growth rate.

DISCUSSION

In vitro fungitoxicity to spores of Cladosporium fulvum

Whereas the substitution of chlorine in the 3-position of salicylanilide virtually eliminated toxicity to spores of *C. fulvum*, in contrast to the enhancement of fungitoxicity by 3-propyl and 3-butyl substituents observed by Topps & Wain (1957), chlorine substitution into the 5- or 3,5-positions caused, approximately, fourfold increases in activity.

The substitution of chlorine into the *para* positions of the anilino (anilide) nuclei of salicylanilide, 5-chlorosalicylanilide and 3,5-dichlorosalicylanilide increased activity by, approximately, 50 %, 300 % and 300 %, respectively. Chlorine substitution into the *para* position of the anilino nucleus did not, however, confer activity on the non-toxic compound, 3-chlorosalicylanilide. It is clear, therefore, that, in this series, the presence of chlorine in both the 5-position of the salicyloyl nucleus and the 4-position of the anilino nucleus is necessary for the exertion of maximum *in vitro* fungitoxic activity towards spores of *C. fulvum*, as assessed by the method indicated.

In vivo fungitoxicity to tomato leaf-mould (Cladosporium fulvum)

Eradicative activity. Of the compounds tested, only two, 3,5-dichlorosalicylanilide and 3,5-dichlorosalicyl-4-chloroanilide, appeared capable of appreciable eradicative action at concentrations non-phytotoxic to uninfected leaf-tissues. However, these two compounds did not affect the normal growth of the fungus when applied to recent infections where the external fungal growth was barely visible, and it was obvious that only where the infected leaf-tissues had been damaged by the spray treatment had the leaf-mould been eradicated. Clearly, these two compounds were more toxic to leaf-tissues extensively penetrated by the fungal mycelium, either because of easier entry into, or through the increased susceptibility of such tissues or, perhaps, following an interaction with metabolic products of the fungus. The destruction of the

mycelium of *C. fulvum* was apparently a secondary effect following on the severe damage to the supporting leaf-tissues

In view of this eradivative activity towards the well-established lesions only, it is surprising that the other compounds, and, in particular, the mildly phytotoxic 3-chlorosalicylanilide, should be virtually inactive as eradicants. It is possible that the effects of 3,5-dichlorosalicylanilide and 3,5-dichlorosalicyl-4-chloroanilide might have been due to their hydrolysis, and the consequent liberation of 3,5-dichlorosalicylic acid, since the effect of a 0.03 % 3,5-dichlorosalicylic acid spray was very similar to those caused by the above two anilides. However, salicylic acid at 0.08 % and 5-chlorosalicylic acid at 0.03 % gave a similar eradication of well-established leaf-mould lesions. Therefore, if hydrolysis was a necessary preliminary for the above toxicity it is apparent that the completely non-eradivative salicylanilide and 5-chlorosalicylanilide were not appreciably hydrolysed by the fungus, or by fungus-invaded leaf-tissues.

Protective activity. Of the compounds tested as protectant fungicides against leaf-mould infection, under conditions favouring severe infection in June and July 1956, only two compounds, 3-chlorosalicylanilide, at 0.06 %, and 3,5-dichlorosalicylanilide, at 0.07 %, gave a good protection. They were, however, phytotoxic at this concentration. 3-Chlorosalicylanilide, at 0.06 %, caused mild leaf-scorch, especially in very hot weather, but did not otherwise affect the plants, whereas 3,5-dichlorosalicylanilide stunted the growth of tomato plants at concentrations exceeding 0.01 %. It is possible that these phytotoxic effects, or some other interaction between chemicals and plants, were responsible for the inhibition of the development of leaf-mould, for 3-chlorosalicylanilide was virtually non-toxic *in vitro* to spores of *C. fulvum* and 3,5-dichlorosalicylanilide was less toxic *in vitro* to *C. fulvum* spores than the 3,5-dichlorosalicyl-4-chloroanilide, which was largely ineffective in the tests on plants.

In view of the relatively high *in vitro* toxicities of several chlorinated salicylanilides, it was surprising that they should have been no more effective, as protectants, than salicylanilide.

In vivo fungitoxicity to cucumber powdery mildew

Eradicative and protective activity. As for tomato leaf-mould, only 3,5-dichlorosalicylanilide and 3,5-dichlorosalicyl-4-chloroanilide were markedly active as eradicants (Table 3). They were, however, phytotoxic.

Again, these two compounds alone gave protection against the disease during a 6-week comparative trial (Table 4). The protection given was, however, incomplete even at phytotoxic concentrations.

The authors wish to thank Miss B. M. Finch for assistance during the course of this work.

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Field trials on the control of club root in Brassicae

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(Received 7 May 1960)

SUMMARY

Experiments were made in 1957-59 on the fungicidal control of club root in cauliflowers (summer and winter), brussels sprouts, summer cabbages and savoys. Plants were treated immediately before setting out or in their final position.

Pure (100 %) calomel used in suspension at 1 and 2 oz. per pint gave best control of the disease: the 4 % calomel paste method was effective but treated plants suffered an initial check from which they only slowly recovered. Formulations of pentachloro-nitrobenzene and of aldrin did not give satisfactory control.

Several methods of applying metham-sodium were tried but gave poor control of the disease.

INTRODUCTION

Club root, caused by the fungus *Plasmodiophora brassicae* (Woron.), is one of the most troublesome and economically the most important disease of cruciferous crops. In Lancashire, especially in the area around Ormskirk, and in Devon and Cornwall, the disease is widely distributed.

The need for a practical chemical method of control has been apparent for a number of years. In the past, market gardeners relied upon the methods developed by Preston (1931) who showed that a 0.05 % solution of mercuric chloride gave fairly good control of the disease when applied at $\frac{1}{2}$ pint per plant after setting out. The disadvantages of this method lay in the poisonous nature of the chemical and the time taken to treat individual plants.

Preston (1931, 1928) also found that when the roots of seedlings were dipped in powdered mercurous chloride (calomel) immediately before planting a beneficial effect was apparent. Further experiments by Preston (1941) showed that the highest level of control was obtained when calomel at 0.6 g. per plant was added either to the dibble hole or along shallow drills. He later (1944) reported effective control of the disease by immersing roots of plants in a calomel paste, prepared by adding 1 lb. of 4 % calomel dust to $\frac{1}{2}$ pint of water before planting. Whitehead (1946) advocated the use of a teaspoonful of 4 % calomel dust in each dibble hole at planting time. Ogilvie (1941)

* Now at the Lea Valley Advisory Unit, N.A.A.S., Cheshunt, Herts.

recommended pure calomel mixed with a convenient carrier, such as sand, dry soil or hydrated lime, applied to dibble holes or along shallow drills.

Preston's calomel paste method has been the standard method of control during recent years. It has gradually gone out of favour mainly because of the check given to the transplants and the difficulties encountered with this material when a planting machine is used.

Mercurous chloride has a low solubility in water (0.00012 % at 18° C., and 0.001 % at 43° C.). For this reason little experimental work has been carried out using this chemical in suspension for the control of club root. Palmer (1941) advocated the use of mercurous chloride, suspended with the aid of gum arabic, at the rate of 5-7.5 lb. per acre applied as $\frac{1}{2}$ pint of the suspension to each plant at planting time.

In view of the consistent and effective control of the disease with the 4 % calomel paste method, but realizing its disadvantages, it was decided to test pure calomel in suspension (2 oz. per pint) as a means of control. At this rate the amount of active material is the same as with the 4 % calomel paste.

Cetas (1958) reported excellent control of club root by the over-all application of 1 quart of 31 % metham-sodium per 100 sq.ft.; approximately 109 gal. per acre. Satisfactory plant growth and good club root control also resulted when metham-sodium was applied as a 16 in. band treatment, 1 pint per 100 ft. length of row; approximately 40 gal. per acre. Treatments were immediately capped by the application of 1-1.5 in. of water from an overhead irrigation line. Planting of cabbages followed 1 month later.

Other materials which had been reported as giving a good control of club root were also tested and compared with the calomel treatment.

GENERAL PROCEDURE

The experiments were conducted on naturally and uniformly infected plots which received normal commercial manurial treatments. Plants were raised in sterilized soil or in land where previous cropping had shown no evidence of club root.

The seedlings received no fungicidal treatment in the seed-bed but were treated immediately before planting or in their final positions.

Aldrin emulsion (0.2 %) was incorporated with each treatment, with the exception of the untreated plots, to give more complete protection against cabbage root fly, *Erioischia brassicae* (Bouché). Where the fungicidal treatment was applied as a dip or a drench, the aldrin solution was prepared first and the appropriate fungicide stirred into the solution. Where the fungicidal treatment was applied to the soil then the transplants were dipped into the aldrin solution before planting.

The pure calomel treatment

A suspension was prepared by adding the required amount (2 oz. or 1 oz.) of pure calomel to 1 pint of water. A handful of plants (25-30) were treated at one time, the roots being dipped in the suspension and stirred round for about 15 sec. to ensure a good cover of the fungicide and to maintain the calomel in suspension.

Plants were set out within half an hour of treatment.

The 4% calomel treatment

The calomel paste was prepared by adding 3 lb. of 4% calomel dust to 1 pint of water. The roots of twelve plants were treated at one time by dipping in the paste to ensure a complete cover of the root system.

Recording of results

Cauliflowers (summer and winter), brussels sprouts, cabbages, and savoys were used as the test crops. These were harvested when mature, and the number and weight of marketable heads were recorded. After cutting, the roots were carefully lifted and graded according to the extent of clubbing:

Key used in Devon experiments

- Grade 0. No apparent club root infection.
- Grade 1. Tap root not clubbed, but lateral roots slightly clubbed.
- Grade 2. Tap root not clubbed but lateral roots moderately clubbed.
- Grade 3. Tap root slightly clubbed, lateral roots severely clubbed.
- Grade 4. Tap root severely clubbed and any laterals present also severely clubbed.
- Grade 5. Plant dead as a result of club root attack.

Key used in Lancashire experiments

- Grade 1. No apparent club root infection.
- Grade 2. Tap root not clubbed, but laterals slightly clubbed.
- Grade 3. Both tap root and lateral roots slightly clubbed.
- Grade 4. Tap root slightly clubbed, lateral roots severely clubbed.
- Grade 5. Tap root severely clubbed and any laterals present also severely clubbed.

*Lancashire**1957 experiments*

The experiment was carried out on a well-drained, deep, light to medium loam, pH 6.9, overlying boulder clay. Six fungicidal treatments were replicated four times on plots of fifty Early Goliath cauliflower plants, hand-planted on 10 May.

The treatments were:

- (i) Roots dipped in a calomel paste—3 lb. of 4% calomel dust to 1 pint of water.
- (ii) 75% wettable pentachloronitrobenzene, $2\frac{1}{2}$ lb. per 100 gal. of water, applied directly to the dibble holes, $\frac{1}{2}$ pint per hole.
- (iii) Roots dipped in a solution of 75% wettable pentachloronitrobenzene, 4 lb. per 100 gal. of water.
- (iv) 20% pentachloronitrobenzene raked into the soil at $\frac{2}{3}$ oz. per sq.yd., approximately 40 lb. per acre P.C.N.B.
- (v) Roots dipped in 0.2% aldrin emulsion.
- (vi) Roots stirred in a pure calomel suspension—2 oz. pure calomel to 1 pint of water. Frequent agitation of the suspension was necessary to prevent the calomel settling.
- (vii) Untreated.

The weather was hot and dry for almost a month after planting but occasional thunderstorms supplied sufficient moisture to establish the plants. Plants that

received the 4 % calomel paste treatment were checked and remained wilted for some time, but recovered after heavy continuous rain. By late July many plants that had not received a calomel treatment were stunted and discoloured. The plants were lifted on 14 August. The results are given in Table 1. The disease index (Table 1) shows that calomel, as paste or suspension, gave a significantly ($P = 0.01$) better control than the other treatments. There was also a substantial increase in the number of heads marketed from the calomel plots. All the pentachloronitrobenzene treatments gave poor control of the disease.

Table 1. *Effect of treatments on the crop 1957*

(Means per plot of 50 plants.)

Treatment	Marketable heads (%)	Plants in infection grades 1, 2, 3 (%)	Total no. of plants	Disease† index
4 % calomel paste*	95.4	92.9	189	2.54
75 % P.C.N.B. (drench) (2½ lb./100 gal.)*	13.1	9.5	190	4.34
75 % P.C.N.B. (dip) (4 lb./100 gal.)*	33.0	21.7	194	4.17
20 % P.C.N.B. (raked in) (40 lb. P.C.N.B./acre)*	24.4	11.7	197	4.44
0.2 % aldrin emulsion (dip)	5.6	4.6	197	4.64
Pure calomel (dip) (2 oz./pint)*	98.0	100.0	200	2.52
Untreated	15.2	11.1	171	4.33
Significant difference ($P = 0.05$)	—	—	—	0.72
($P = 0.01$)	—	—	—	1.11

* 0.2 % aldrin was incorporated with these treatments.

† Disease index: The summation of the grades into which individual root systems from each treatment were classified, divided by the total number of root systems assessed.

A positive correlation (0.82) was shown between the number of marketable heads cut from any treatment and the sum of the plants falling in grades 1, 2 and 3 in that treatment. This would indicate that under the conditions of this experiment only plants which showed slight clubbing of the tap root would form a marketable head.

Devon

Concurrently, a trial along similar lines was carried out at Northam, Devon, where fungicidal treatments were replicated three times on plots of thirty plants of winter cauliflower. The soil was a deep sandy loam, pH 6.7. Treatments were applied and the area planted on 12 August.

The treatments were largely the same as those used in the Lancashire experiment, except that the 75 % wettable pentachloronitrobenzene, 4 lb. per 100 gal., was applied at ½ pint per plant and not as a dip, and the 20 % pentachloronitrobenzene treatment was replaced by 0.05 % mercuric chloride applied to the dibble holes, ½ pint per plant.

The weather after planting favoured plant establishment. Plants treated with 4 %

calomel paste and with 0.05 % mercuric chloride were checked and recovered slowly. Cutting of heads began on 28 February; when this was completed root assessments were made with the results shown in Table 2.

There was some rabbit damage to plants treated with 0.05 % mercuric chloride.

The results indicate a superior control of the disease by the mercury treatments. The pure calomel treatment showed a significant ($P = 0.05$) increase in the weight of head compared with the control plot.

Table 2. *Effect of treatments on the crop 1957*

(Means per plot of 30 plants.)

Treatment	Weight of head (oz.)	Disease index
4 % calomel paste*	10.60	1.70
Pure calomel (dip) (2 oz./pint)*	11.60	1.70
75 % P.C.N.B. (drench) (4 lb./100 gal.)*	8.50	2.80
0.05 % mercuric chloride (drench)*	7.00	1.71
0.2 % aldrin emulsion (dip)	9.55	2.93
Untreated	9.84	3.12
Standard error per plot	1.20	0.46
Significant difference ($P = 0.05$)	1.41	0.54

* 0.2 % aldrin was incorporated with these treatments.

Lancashire

1958 experiments

The treatments used were similar to those in 1957, except that the 75 % wettable pentachloronitrobenzene (2½ lb. per 100 gal., ½ pint per plant) was replaced by a pure calomel treatment, 1 oz. per pint. Brussels sprouts were planted at Melling near Ormskirk on 6 May. The treatments were replicated four times on plots of twenty-eight plants.

Table 3. *Effect of treatments on the crop 1958*

(Means per plot of 28 plants.)

Treatment	Average weight (lb.)	Disease index
4 % calomel paste*	2.74	2.99
Pure calomel (dip) (1 oz./pint)*	3.52	2.49
Pure calomel (dip) (2 oz./pint)*	3.79	2.20
75 % P.C.N.B. (dip) (4 lb./100 gal.)*	2.95	3.35
20 % P.C.N.B. (raked in) (60 lb. P.B.N.C./acre)*	2.84	3.41
0.2 % aldrin emulsion (dip)	2.87	3.23
Significant difference ($P = 0.05$)	0.55	0.58
($P = 0.01$)	0.75	0.80

* 0.2 % aldrin was incorporated with these treatments.

Plants which received the 4 % calomel showed an initial check and were retarded for about 1 month from planting. Lifting was carried out on 29 October, the roots were graded for club root infection and the above-ground parts of the plant weighed. The results are shown in Table 3.

Both the pure (100 %) mercury treatments gave good control of the disease, significantly ($P = 0.05$) better than other treatments. The pure calomel treatment at 2 oz. per pint, gave a significantly ($P = 0.05$) better disease control than the 4 % calomel. The control of the disease given by the pure calomel suspensions was reflected in the increased weight per plant, significantly ($P = 0.05$) higher than from other treatments. The poor yield shown by the 4 % calomel paste treatment was probably due to the initial check suffered by the plants after setting out.

Devon

In view of the successful results reported by Cetas (1958) it was decided to test the effectiveness of metham-sodium in the south-west. Metham-sodium applied as a drench and as a band was compared with 4 % formaldehyde. Treatments were duplicated and applied on 21 May on a sandy loam soil (pH 6.3) at Northam, North Devon, as follows:

- (i) 31 % metham-sodium at approximately 90 gal. per acre applied as a drench: $\frac{1}{2}$ pint metham-sodium in $\frac{2}{3}$ gal. of water per square yard.
- (ii) 31 % metham-sodium at 90 gal. per acre applied to the drill: $\frac{1}{2}$ pint metham-sodium in 1 gal. of water to a 12 in. band, 30 ft. long.
- (iii) 4 % formaldehyde applied at $\frac{2}{3}$ gal. per sq.yd.
- (iv) Untreated.

Extra Early broccoli were planted on 25 June in plots of eighty-four plants. Cutting began on 9 October; the results are given in Table 4.

Table 4. *Effect of treatments on the crop 1958*

(Means per plot of 84 plants)

Treatment	No. of marketable heads	Weight of head (oz.)	Disease index
4 % formaldehyde	49.00	12.58	4.02
Metham-sodium (drill)	43.50	11.80	4.06
Metham-sodium (drench)	40.50	10.15	4.26
Untreated	26.50	6.40	4.79
Significant difference ($P = 0.05$)	12.40	2.98	0.55

The 4 % formaldehyde gave the greatest number of marketable heads and the highest yield. All treatments gave initial protection against the disease and gave a significantly ($P = 0.05$) higher number of marketable heads in comparison with untreated plots but no treatment gave good control of the disease throughout the season, although the 4 % formaldehyde and metham-sodium applied to the drills were significantly ($P = 0.05$) better than the untreated plots.

Metham-sodium was further tested in the control of club root on dwarf Essex rape. Treatments were the same as before, except that the 4 % formaldehyde was replaced by metham-sodium used as a concentrated solution (approximately 86 gal. 31 % metham-sodium applied in 86 gal. water per acre).

Treatments, replicated three times were applied at Starcross on 18 June. Seed was sown on 27 August and assessments made on 24 October with the results shown in Table 5.

Table 5. *Effect of treatments on the crop 1958*

Treatment	Plants infected (%)	Disease index
Metham-sodium (concentrated)	51.54	1.92
Metham-sodium (drill)	29.32	1.06
Metham-sodium (drench)	56.83	2.09
Untreated	81.36	3.23
Significant difference ($P = 0.05$)	49.76	0.63

The number of plants infected in all treatments was high. The plots which received the drill application showed significantly ($P = 0.05$) lower percentage infection than control and gave a significantly ($P = 0.05$) better disease control than other treatments.

Lancashire

1959 experiments

The experiments in 1957 and 1958 had been carried out on mineral soils in the pH range 6.5–7.5. Club root also seriously affects brassica crops on the moss soils of Lancashire.

Two rates of pure calomel (2 oz. and 1 oz. per pint) were compared with 4 % calomel paste on moss soil (pH 5.7) at Rainford, Lancashire. Treatments were replicated five times on plots which contained sixty Early Goliath cauliflower plants, spaced at 2 ft. $\times$ 2 ft.

A representative soil sample from the experimental area planted with cabbage seedlings (Colhoun, 1957) showed 100 % infection of the plants with club root after 5 weeks. Planting was carried out by hand on 6 May. Plants that received the 4 % calomel treatment were checked in the early stages of growth but recovered slowly. Visual assessments showed no apparent differences between the treatments until 8 July when plants in the untreated plots showed permanent wilting. Cutting of heads began on 20 July; the results are given in Table 6.

Table 6. *Effect of treatments on the crop 1959*

(Means per plot of 60 plants.)

Treatment	No. of marketable heads	Disease index
Pure calomel (dip) (2 oz./pint)*	45.2	2.46
Pure calomel (dip) (1 oz./pint)*	43.0	2.64
4 % calomel paste*	45.0	2.38
Untreated	12.2	4.73
S.E. per plot	5.01	0.12
Significant difference		
($P = 0.05$)	6.90	0.17
($P = 0.01$)	9.68	0.24

* 0.2 % aldrin was incorporated with these treatments.

The pure calomel (2 oz. and 1 oz. per pint) and the 4 % calomel treatment gave very good control of the disease on heavily infected acid soil. The 4 % calomel paste treatment gave significantly ($P = 0.05$) less disease than the pure calomel treatment used at the 1 oz. per pint rate but with no significant increase in yield. The number of heads marketed from all treated plots was significantly ($P = 0.01$) higher than from the untreated plots.

Devon

A similar experiment was carried out at Northam, Devon. The same treatments were used, and the aldrin treatment ($\frac{1}{2}$ pint per plant of 0.04 % aldrin emulsion) developed by Channon & Keyworth (1960) was incorporated.

Each treatment was replicated eight times on plots which consisted of twenty savoy and ten cabbage plants. Treatments and planting were carried out on a deep coarse sandy loam, pH 6.4, on 14 July. The soil was extremely dry and warm at planting time and plant establishment was assisted by overhead irrigation. Frequent visual assessments showed wilting and stunting of cabbage plants in all plots 1 month after planting, except those treated with calomel. Wilting in the same treatments occurred in the savoy plots 3 weeks later.

Table 7. *Effects of treatments on the crop 1959*

Treatment	Savoy (means per plot of 20 plants)			Cabbage (means per plot of 10 plants)		
	No. of marketable heads	Weight of head (oz.)	Disease index	No. of marketable heads	Weight of head (oz.)	Disease index
Pure calomel (dip) (2 oz./pint)*	11.88	14.62	3.01	6.75	13.65	2.04
Pure calomel (dip) (1 oz./pint)*	11.25	14.02	3.13	5.50	11.45	2.15
4 % calomel paste*	9.25	14.25	3.55	5.63	9.84	3.05
0.04 % aldrin emulsion (drench)*	5.86	8.83	4.34	4.00	8.85	3.75
Untreated	1.13	3.82	4.70	1.25	4.25	4.03
Standard error per plot	3.83	3.89	0.35	1.29	2.29	0.38
Significant difference ($P = 0.05$)	3.91	3.97	0.39	1.33	2.25	0.39
($P = 0.01$)	5.28	5.36	0.53	1.80	3.16	0.49

* 0.2 % aldrin was incorporated with these treatments.

Cutting of cabbages began on 9 September and savoy 4 weeks later.

The results are shown in Table 7. The pure calomel (2 oz. and 1 oz. per pint) and the 4 % calomel treatments gave good control of the disease. The figures of disease index per plant, in the savoy experiment, are significantly ($P = 0.05$) lower for the pure calomel 2 oz. per pint, than for the 4 % calomel plots. The disease indices from the cabbage experiment show that both rates of pure calomel gave a significantly ($P = 0.01$) better control than the 4 % calomel treatment. Similar trends are apparent when the number of heads marketed and weight of heads from the cabbage experiment are examined. The aldrin treatment gave poor control of the disease.

Cost of the calomel treatments

The approximate costs of the calomel treatments used are as follows:

Treatment	Cost of 1 lb. material	No. plants treated per pint	Cost per acre (10,000 plants)
2 oz./pint pure calomel	26s.	500	£3. 5s. 0d.
1 oz./pint pure calomel	26s.	500	£1. 12s. 6d.
4 % calomel 3 lb./pint	2s. (1 cwt., 226s.)	300	£10. 0s. 0d.

DISCUSSION

The results of the trials carried out over 3 years show that a practical control of club root can be obtained by the use of pure calomel in suspension at 1 oz. and 2 oz. per pint. The control is not complete, but the treatments delay initial infections and enable treated plants to produce a high proportion of marketable heads. No check to growth was noted in any of the treatments. The pure calomel treatments are relatively cheap; at the 2 oz. per pint rate the cost of the treatment is approximately one-third that of the 4 % calomel paste method. It is possible to treat a large number of plants in a short time using this method, which is particularly convenient when a planting machine is used. A further advantage of the treatment is that aldrin emulsion can be added to the calomel suspension to give more complete protection against cabbage root fly.

The calomel is prevented from settling by frequent agitation, which is accomplished by constantly swirling the roots of the plants in the suspension. Dilution of the suspension by soil may result when large numbers of plants are treated; to prevent this each 1 pint of suspension should be replaced after treating approximately 600 plants: alternatively the roots of the transplants should be washed by dipping in water before treatment.

The 4 % calomel paste treatment also gives a good control of the disease, but the plants suffer an initial check and their recovery is usually slow. This effect is particularly evident in the 1958 Lancashire experiment and is reflected in the data on weight per plant. The check to growth is most noticeable when treated plants are exposed to hot, dry weather, when the calomel paste dries out to form a hard crust surrounding the root system and thus restricts subsequent root development. A further disadvantage of the 4 % calomel treatment is the difficulty encountered at planting time when a planting machine is used.

The 0.05 % mercuric chloride, 0.04 % aldrin emulsion and the 75 % pentachloronitrobenzene treatments necessitate the application of the fungicide as a drench to individual plants. Even if these fungicides provide effective control of the disease they would be unacceptable to the market gardener because of the labour and time required to administer the treatments by hand. Alternatively, their application necessitates the purchase of expensive and specialized planting machines.

The pentachloronitrobenzene treatment is ineffective. Its high cost and the labour required for its application would make it unpopular with the vegetable grower.

Metham-sodium failed to give a good control of the disease. The objectionable properties of the material; the fact that water is required in fairly large quantities to ensure adequate wetting of the soil, and the time required to elapse between treatment and planting are drawbacks to its use.

It is considered that the pure calomel treatment is practical, economic and effective and merits use by market gardeners. On crops to be planted in land where low levels of contamination are suspected, or when applied as a routine precautionary treatment, pure calomel at 1 oz. per pint is recommended. Where heavily infected land has to be cropped, pure calomel at 2 oz. per pint should be used.

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***Verticillium* wilt of tomatoes—the role of pectic and cellulolytic enzymes**

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(Received 1 July 1960)

SUMMARY

An isolate of *Verticillium albo-atrum*, highly pathogenic to tomato plants, secreted polygalacturonase (PG) on a variety of synthetic media. Particularly active filtrates were obtained from cultures on media containing mineral salts, casamino acids and pectin or polypectate. Pectates were degraded more rapidly than pectin. None of the filtrates had high cellulase or pectinesterase activity; the most active were from cultures with carboxymethyl cellulose or pectin as carbon sources.

The fungus made little growth in exudates from xylem sap unless carbon sources were added, and then culture filtrates had appreciable PG and cellulase activity but very little pectinesterase activity. Saps from susceptible and resistant varieties gave similar results.

The fungus was also grown on a synthetic sap based upon an analysis of xylem exudate. Results similar to those for xylem sap were obtained.

Filtrates from cultures on dead stems of both varieties had little pectinesterase activity and a definite but relatively low PG and cellulase activity.

Extracts from diseased plants had no PG or cellulase activity but had a considerably higher pectinesterase activity on fresh and dry weight bases. Exudates from xylem of diseased plants had no PG or cellulase activity but had a low pectinesterase activity which was slightly higher than that from healthy plants.

The effects of different culture filtrates on cuttings is described. Filtrates with high PG activity produced some of the symptoms found in naturally diseased plants.

Solutions of pectin, polypectate and carboxymethyl cellulose caused cuttings to wilt in very low concentrations; much higher concentrations were needed after solutions of these substances had been degraded by PG or cellulase.

INTRODUCTION

There are still comparatively few studies which deal with the infection of plants by *Verticillium* spp., and with the ways in which symptoms of disease are produced after the fungus has passed into the xylem of the shoot and grown there for some time. It has been assumed that the fungus secretes into the tracheal fluids substances which damage the plant in one way or another, but there is no agreement about the nature

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and activity of these substances. In the past few years evidence has been obtained which suggests that they may be extracellular enzymes, particularly those which act upon cell walls (Scheffer, Gothoskar, Pierson & Collins, 1956), toxins in the more restricted use of the word (Talboys, 1957), or growth-regulating substances (Pegg & Selman, 1959). It is possible, of course, that all three types of substance, as well as others, may be involved.

This paper deals with the possibility that pectic and cellulolytic enzymes are produced in the xylem of infected plants and cause some of the symptoms found in diseased plants.

MATERIALS AND GENERAL METHODS

(a) *Tomato plants*, variety Bonny Best, were used in most of the experiments. The resistant variety Loran Blood was also used occasionally. They were grown in sand watered with a solution containing (p.p.m.): Ca, 400; Mg, 96; K, 468; N as NO_3 , 420; P as PO_4 , 62; S as SO_4 , 128; Zn, 0.02; Bo, 0.02; Mo, 0.032; Fe, 2.9; Mn, 0.2; Cu, 0.028.

Plants were grown in a well-lit greenhouse where the temperature was generally in the range 24–27° C. and did not go below 24° C. After they had been inoculated with the pathogen they were kept in a house in which the temperatures were some 6° C. lower because the pathogen did not produce good symptoms of disease at the higher temperatures.

(b) *The pathogen* was originally isolated from a tomato plant with typical *Verticillium* wilt symptoms. It was provisionally identified as *Verticillium albo-atrum*. Later, Dr Isaac, University College, Swansea, kindly examined it and described it as a slightly atypical specimen of this species. Stock cultures were maintained on potato-dextrose agar under mineral oil. Liquid media, generally in 500 ml. Erlenmeyer flasks, were inoculated at the rate of 1 ml. spore suspension, containing *c.* 1×10^6 spores, per 100 ml. medium. Cultures were grown for 14 days at room temperature, generally about 22–24° C.

Microsclerotia did not appear in any of the cultures and with few exceptions dark-coloured hyphae appeared only in old cultures. Typical spores were produced abundantly on a variety of media.

(c) *Cultures*, grown on a liquid medium, were used to inoculate plants. The medium contained: sucrose, 1.5 %; NaNO_3 , 0.2 %; KH_2PO_4 , 0.1 %; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 %; yeast extract, 0.02 %; 100 ml. was placed in 500 ml. Erlenmeyer flasks, and the fungus was harvested on nylon cloth after it had grown for 14 days. The mats of mycelium were washed in water and then comminuted in a Waring Blendor for 15 sec. in a volume of water twice that of the original culture.

A ring of sand about 25 mm. deep, 25 mm. wide, and inner circumference 25 mm. from the tomato plant was removed and a quantity of suspension corresponding to half of one of the original cultures was poured into the hollow and the sand was then replaced. This method of inoculation was always successful with the susceptible variety. The first symptoms of disease, pronounced epinasty, appeared in about 7 days, and other well-defined symptoms appeared in the following 2–3 weeks.

(d) The following *pectic and cellulosic materials* were used:

(1) High methoxyl pectin and sodium polypectate kindly supplied by Sunkist Growers, Ontario, California. They were washed in 60% ethanol containing 0.1N-HCl, followed by 95% ethanol until chloride was not detected visually by the silver nitrate test, and then dried at 60° C. before use.

(2) Carboxymethyl cellulose (CMC) was kindly supplied by the Hercules Powder Co.; a sample with a degree of substitution (DS) of 0.4 was used in culture media, and one with a DS of 0.7 was used in viscosity tests.

(3) Wood cellulose (Solka Floc) was given by the Brown Company, Boston, Mass. It was a highly purified and finely divided product stated to consist of at least 99.5% cellulose.

(e) The activity of enzymes which attack the main chains of pectin and pectates (polygalacturonase—PG), or CMC (cellulase—Cx *sensu*, Siu & Reese, 1953) in a random manner may be estimated by measuring the rate at which these enzymes reduce the viscosity of solutions of these substances. Test solutions finally contained 0.75% pectic substances or 0.5% CMC, HCl or NaOH to bring the pH to the required value, phosphate or citrate buffer (final concentration 0.02M), and 10% enzyme solution. Viscosity was measured at 30° C. with an Ostwald type viscometer 6, 12 and 20 min. after adding the enzyme.

Activity of PG and cellulase, expressed as the inverse of the time required to produce given viscosity changes, was found to be directly proportional to enzyme concentration.

(f) Pectinesterase activity was estimated by titrating with dilute NaOH the COOH groups produced when ester linkages in solutions of high methoxyl pectin were hydrolysed. Test solutions contained 1% pectin, 0.2M-NaCl, HCl or NaOH to bring the solution to a suitable pH, 1% standard solution of bromo-cresol green or bromothymol blue, and enzyme solution. The pH of the mixture was not allowed to move more than 0.5 unit from the initial value.

EXPERIMENTAL

Growth and enzyme secretion in vitro

(a) *On standard culture media*

In a number of experiments the fungus was grown in a basal medium containing KH_2PO_4 , 0.1%; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05%; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 p.p.m.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1 p.p.m.; $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 p.p.m.; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.15 p.p.m., to which was added various sources of nitrogen and carbon; 100 ml. of the media in 500 ml. Erlenmeyer flasks were seeded and incubated in the way described above. The amount and type of growth was recorded, the culture was filtered through nylon cloth, and cells and debris were removed from the filtrate by centrifuging at 10° C. Filtrates were frozen at -20° C., and enzyme activity was measured later, and within a day of thawing. During the interval between thawing and testing, solutions were kept under toluene at 5° C. The main points which emerged from the results of a number of experiments are summarized below.

(1) *Growth*. So long as the pH of media did not drift to unfavourably low values,

growth was not very much affected by the use of different nitrogen sources. Nitrates and casein hydrolysate supported good growth when the carbon source was suitable; ammonium salts were less satisfactory unless the drift to acid conditions which they caused were prevented by the addition of excess CaCO_3 . When other conditions were suitable the fungus grew well on glucose and CMC, formed a thick surface mat, and sporulated profusely. There was considerably less growth on sodium polypectate and on pectin. Typical dry weights of mycelium per flask with casein hydrolysate (0.033 % N in medium) as the nitrogen source, and 1 % carbon source were: glucose, 156 mg.; CMC, 129 mg.; sodium polypectate, 87 mg.; pectin, 48 mg. The initial pH of these media had been adjusted to 7.0, and the final pH was in the range 7.7-8.3, so it is unlikely that these differences in growth had been caused by different pH conditions in the cultures.

When wood cellulose was used it was not possible to determine weights of mycelium because of the undigested cellulose which remained, but it appeared that growth was always very poor.

(2) *Enzyme activity.* The PG in culture filtrates degraded pectin solutions most rapidly at pH 4.5-5.0, but on polypectate solutions activity was highest at pH 5.5-6.0.

Filtrates with the highest PG activity were generally obtained from cultures grown on pectin or polypectate, but enzyme secretion was not wholly adaptive, because quite active filtrates were sometimes obtained when glucose or CMC was the carbon source. Filtrates from cultures on wood cellulose always had a very low activity.

Table 1. *Effect of different N sources on secretion of PG*

Carbon source	Nitrate		Casamino acids	
	% reduction viscosity after			
	20 min.		6 min.	
	Pectin	Polypectate	Pectin	Polypectate
0.4 % glucose	10	75	10	63
1.0 % glucose	3	45	23	89
0.4 % pectin	30	94	74	97
1.0 % pectin	35	97	47	97
0.4 % CMC	20	89	23	91
1.0 % CMC	10	80	10	83

Casamino acids and sodium nitrate produced similar pH changes in media containing the same quantity of nitrogen so that they were useful for comparing the effect of different sources of nitrogen on enzyme secretion. When they were used at a final concentration of 0.033 % N with 0.4 or 1 % glucose, pectin or CMC in the basal medium it was found that the two levels of carbon source had no consistent effect on the activity of the culture filtrates, but that casamino acid culture filtrates were more active than were those containing nitrate. This is shown in Table 1, which shows the percentage change in viscosity of pectin and polypectate solutions by cell-free filtrates. The tests were done at pH 6.5 which is not far from the optimum pH for the degradation of polypectate, but is considerably above the optimum pH for the degradation of

pectin. This in part explains differences in activity of the same culture filtrates on the two substances. The data show that with one exception the casamino acid filtrates produced as much or more change in viscosity in 6 min. as did the nitrate filtrates in 20 min., i.e. they were at least three times as active.

The effects of a diluted culture filtrate (carbon source polypectate) on solutions of pectin and polypectate, at pH values near the optima for these two substances, are shown in Table 2.

Table 2. *Effect of culture filtrates on solutions of pectin and polypectate*

	% reduction of viscosity after			pH
	6 min.	12 min.	20 min.	
0.75 % pectin	41	58	68	5.0
0.75 % polypectate	72	85	90	6.0

Table 3. *Cellulase activities of culture filtrates*

Carbon source	% reduction of viscosity after		
	6 min.	12 min.	20 min.
Glucose	12	21	30
Pectin	2	3	5
Sodium polypectate	2	3	5
CMC	77	80	85
Wood cellulose	4	13	25

Polypectate was degraded about three times as rapidly as was pectin.

In the light of the above and other results, 1 % pectin or polypectate, and 0.4 % casamino acids were used in the basal salts medium when culture filtrates with high PG activity were required.

Only in one of a considerable number of experiments were reasonably high cellulase activities obtained. Filtrates from cultures with 1 % carbon source and 0.033 % N as NaNO_3 had the activities shown in Table 3; pH of the mixtures was 7.0.

The pectin and polypectate filtrates were almost inactive, the glucose and wood cellulose filtrates had a slight but definite activity. The CMC filtrate was much more active but the activity was low compared with the PG of appropriate filtrates on pectin or polypectate.

In other experiments, much lower cellulase activities were recorded, the differences between glucose and CMC filtrates were not so great, and wood cellulose filtrates were almost inactive. This was surprising in view of the fact that the fungus did make some growth on this insoluble material, but it was perhaps more surprising that the CMC filtrates were not more active, because the fungus grew almost as well on this substance as on glucose, and presumably must have degraded it considerably before it was assimilated. This aspect of enzyme secretion would repay further study because the cellulolytic activity of a fungus is often assessed on the activity of filtrates from cultures on appropriate substrates. Clearly this will be misleading if the substrate of

the enzyme is degraded extensively during growth of the fungus although the enzyme does not accumulate.

In a single experiment in which enzyme secretion in media containing sodium nitrate and casamino acids was compared, one was found to be as good as the other.

Cellulase activity was highest in the range 5.5–7.0 with no well-defined optimum.

When the pectinesterase activities of cultures on different carbon sources were compared it was found that filtrates from cultures on glucose, polypectate, CMC or wood cellulose were less active than those from pectin cultures, but even the most active had a relatively low activity, causing no more than a 10% hydrolysis of available ester linkages in 2 hr. at pH 6.5, and under the other conditions described above.

(b) *In xylem exudates*

(1) *Growth.* Most of the characteristic disease symptoms are produced while the fungus is confined to the xylem elements. It was of interest, therefore, to see how well the fungus grew and secreted enzymes in tracheal sap. This was obtained from 7-week-old Bonny Best and Loran Blood plants by cutting the main stem about 25 mm. above the level of the sand, and fitting it with a rubber tube holding a 10 ml. pipette vertically. Sap passed from the cut end into the pipette, was collected daily for 3–4 days and stored at -20°C . Each day, the pipette with the exudate was removed and the cut surface of the shoot and the rubber tubing were well washed with distilled water before a clean pipette was put in place. Twelve plants of each variety were used. Although they had been grown side by side in the greenhouse and were similar in size and appearance, they exuded sap at very different rates. Hardly any was obtained from seven of the Loran Blood plants and from five of the Bonny Best plants, and two or three plants of each variety accounted for much of the sap that was collected.

Over the 4 days 160 ml. of sap was obtained from the Bonny Best plants, and 112 ml. from the Loran Blood plants. The pH of both saps was initially 5.8, rising later to 6.6

Table 4. *Growth of Verticillium albo-atrum in xylem sap*

Additional carbon source (0.2 %)	Dry wt. (mg.) mycelium/culture			
	Bonny Best		Loran Blood	
	(1)	(2)	(1)	(2)
None	1.6	1.7	1.1	1.3
Glucose	9.2	7.3	7.1	8.7
Pectin	5.8	3.9	3.1	5.6
Sodium polypectate	—	6.3	—	8.3
CMC	5.4	3.7	3.1	3.8

14 ml. of the exudates was placed in 100 ml. Erlenmeyer flasks with 28 mg. glucose, pectin or CMC, or with no additional carbon source. The pH of each solution was adjusted to 6.5 with a few drops of NaOH solution before autoclaving; the flasks were inoculated with 0.5 ml. water containing about 5×10^5 spores, and incubated at 20 – 22°C . for 10 days. The mycelium was harvested and the culture filtrates were stored at -20°C .

This experiment was repeated some 3 months later when much better yields of sap were obtained. Again, different plants gave very different volumes of sap, but 240 ml. was obtained from Bonny Best plants in 3 days and 255 ml. from Loran Blood plants. The pH values were somewhat higher, initially 6.0 and finally 7.0. The dry weights of mycelium in Expts. 1 and 2 are shown in Table 4.

The final pH of all cultures was in the range 7.7–8.3. The fungus grew poorly in the xylem sap presumably because of the shortage of carbon, because when glucose was added growth was much increased. The fungus was also able to use pectin, polypectate and CMC under these conditions, and the growth on polypectate was almost as good as that on glucose, and about twice that on CMC. In this respect the results differed from those described earlier in which the fungus always grew better with CMC. Although the results of the first experiment suggested that the fungus grew better in Bonny Best sap, this was not confirmed in the second experiment in which the opposite results were obtained. Any real differences caused by differences in the composition of the saps are outweighed by the much greater effects of the added carbon sources.

(2) *Enzyme activity.* The enzyme activities of each of the culture filtrates were measured in the ways already described. All tests were done at pH 6.5–7.0, values close to those of the xylem saps.

The filtrates from cultures on Bonny Best and Loran Blood saps had very similar PG activities so they will be dealt with together. None of the filtrates caused a significant reduction of the viscosity of pectin solutions in 20 min. Polypectate solutions were degraded most rapidly by the filtrates from pectin cultures which caused a 55 % reduction in viscosity in 20 min.: corresponding figures for the polypectate and CMC culture filtrates were 22 and 29 %.

Only cultures grown on CMC significantly reduced the viscosity of CMC solutions in 20 min.; 83 % of the total possible change took place in this period so that these filtrates were about as active as the best of those from the synthetic media which had supported much more profuse growth of the fungus. Pectinesterase activity of all filtrates was very low. Pectin cultures were the most active, but even these caused only about 2 % hydrolysis of methyl ester groups in 3 hr. The activity of the other filtrates was one-sixth to one-third of this.

These experiments showed that the fungus grew very little in the saps from the xylem of Bonny Best and Loran Blood plants, and produced very little of the enzymes which act upon pectin, polypectate or CMC. When these substances were added to the sap, a fair amount of growth was made, and quite readily detectable amounts of chain-splitting enzymes were secreted.

(c) *In synthetic sap*

Samples of sap collected from Bonny Best and Loran Blood plants during the first 24 hr. after cutting the stem were very kindly analysed by Dr Fisher of the Connecticut Agricultural Experiment Station. The results in p.p.m. were as shown in Table 5.

Analysis by chromatography showed that both types of sap contained similar

quantities of at least seven substances reacting to give coloured products with ninhydrin. Substances containing NH_3 and NO_3 were also present, but preliminary analyses showed that these accounted for less than 50% of the total nitrogen present.

Chromatography showed only traces of substances reacting with carbohydrate and phenol reagents.

On the basis of these results and on the findings of Bollard (1953) that most of the nitrogen in xylem sap from plants is in organic form, a synthetic sap was prepared in which the nitrogen was supplied as vitamin-free casamino acids.

Table 5. *Analysis of fluids from xylem of tomato plants*

Substance	Variety		Substance	Variety	
	Bonny Best	Loran Blood		Bonny Best	Loran Blood
Total solids	1400	972	Sodium	3.9	2.6
Total ash	967	664	Manganese	0.63	0.44
Nitrogen	98	48	Iron	0.57	0.74
Calcium	151	103	Aluminium	0.20	0.12
Magnesium	27	19	Copper	0.21	0.16
Phosphorus	44	30	Boron	0.69	0.46
Potassium	206	131			

Calcium and sodium were supplied as the chloride, magnesium and minor elements as the sulphate, potassium and phosphorus as tribasic potassium phosphate. The final concentrations conformed as closely as possible to those in Table 5. In addition, carbon sources were supplied as pectin, polypectate, CMC, or wood cellulose at concentrations of 0.2 and 0.04%, and cultures were grown in these media in the way described for xylem exudates. The results obtained with 0.2% carbon source were generally similar to those obtained with the natural sap and added carbon. Each of the filtrates was almost inactive on pectin, but the pectin and polypectate filtrates were about equally active on polypectate solutions causing a 50% reduction of viscosity in about 20 min. Only the CMC cultures were active on CMC solutions; again the viscosity was reduced by about 50% in 20 min. The pectinesterase activity of each of the filtrates was negligible.

Cultures on the media containing only 0.04% of the carbon sources had very little enzyme activity.

Growth and enzyme secretion on dead stems

Although the fungus is normally confined to the xylem elements while the plants are still alive, it was thought worthwhile to examine growth and enzyme secretion on stems, and on petioles below the level of the first leaflets. These were collected from 8-week-old plants and cut into 2 cm. lengths; 7.0 g. were placed in 250 ml. flasks, some of which were put into an autoclave already at 100° C., and then autoclaved at 10 p.s.i. for 5 min. and cooled as quickly as possible afterwards. 1 ml. propylene oxide was put in the other flasks which were plugged with cotton-wool, and made airtight for 24 hr. The seals were removed and the propylene oxide was allowed to disperse; 40 ml. sterile water was added to each of the flasks which were inoculated with 0.5 ml.

spore suspension (about 5×10^5 spores) and incubated for 2 weeks at 20–22° C. The fungus grew as well on Bonny Best as on Loran Blood stems. When the cultures were shaken, only the vascular cylinders remained coherent, the rest of the tissue disintegrating completely. This, and the good growth of the fungus, suggested that the insoluble material of the cell wall had been degraded considerably. Filtrates were obtained by removing particulate matter by filtering and centrifuging, or after the whole culture had been treated in a Waring Blendor for 15 sec., NaCl added to a final concentration of 0.2M and the pH adjusted to 8.0 and kept for 1 hr. before obtaining the cell-free filtrate. The second series of treatments was applied to release enzymes adsorbed by cell-wall material. None of the filtrates had any appreciable activity on pectin, and caused no more than a 50% reduction of viscosity of polypectate and CMC solutions in 20 min.; pectinesterase activity was also very low. This result agrees with those of other experiments in which it was found that although sufficient enzyme to degrade the substrate in the media must have been produced, culture filtrates had very little enzyme activity.

Pectic and cellulolytic enzymes in healthy and diseased plants

If pectic and cellulolytic enzymes are produced in diseased plants then, unless they are inactivated, it should be possible to detect them, because the methods used for measuring their activity can be made very sensitive. Accordingly two lots of plants, grown and inoculated at different times of the year, were examined for these enzymes.

In the first experiment, Bonny Best plants were grown at 24–27° C. until they were about 12 in. tall. They were transferred to a greenhouse at about 18° C., half were inoculated in the way already described, and the rest were treated in the same way except that no fungus was used. After 7–10 days there was pronounced epinasty in all inoculated plants, the lower surface of the petiole often making an angle as low as about 45° with the main stem. After about 12–14 days the lower and middle leaves of inoculated plants were wilting and later the disease symptoms intensified so that after 28 days inoculated plants were only about a third to a half as high as the controls, some of the lower leaves had been shed, and all other leaves except those of the top shoot were wholly or partially desiccated. Internally, there was pronounced vascular browning beyond the node of the topmost, fully expanded leaf, and in the petioles of all leaves left on the stem. Uninoculated plants grew normally.

Four weeks after inoculation the shoots of four plants, taken at random from each group, were cut at the level of the sand, and the fresh weights of the main stems, petioles, and leaflets were determined. Each portion was ground in a mortar and then comminuted in a high-speed homogenizer with very sharp blades for 2–3 min. The mush was filtered through fine-mesh nylon cloth and the filtrate centrifuged at 4000 r.p.m. at 5° C. for 15 min. The supernatant was collected and made up to a known volume. The precipitate was added to the material retained by the nylon cloth and extracted with 0.2M phosphate at pH 8.0 under toluene at 4° C. overnight. The mixture was centrifuged as before and the supernatant solution was made up to a known volume.

Portions of the water and phosphate extracts were dialysed under toluene at 4° C. overnight in 20 times their volume of 0.01 M-NaCl, and again for 6 hr. in a fresh solution. Changes of volume after dialysis were noted, and then all extracts were stored at -20° C. until enzyme activities were measured. During these operations, as far as was practicable, extracts were kept under toluene at 4° C. before they were frozen.

In the second experiment, the plants were used 18 days after inoculation, and half of the uninoculated plants were not watered for the last 3 days so that they were completely wilted when the shoots were cut. The shoots were not divided into different parts as in the first experiment, but were finely chopped, and divided into two approximately equal portions. The fresh weights of each were determined. One of the two portions was then dried at 70° C. for 48 hr. to determine dry weight, and the other was extracted in 0.2 M phosphate buffer at pH 8.0. Afterwards the procedure was as before.

The activity of viscosity-reducing enzymes was determined in the ways already described except that the mixtures were kept for long periods under toluene at 20-22° C. so that traces of enzymes present in the extracts would be detected.

When polypectate or pectin was used for determining PG activity, none of the extracts from diseased plants behaved differently from the corresponding extracts from uninoculated plants. The viscosity of the solutions often increased after incubation with the extracts, presumably because certain cations were present which reacted with the polypectate, and with the pectate formed from the pectin by the pectinesterase which was present in each of the extracts.

Table 6. *Pectinesterase activity of diseased and uninoculated plants*

Part of plant	% hydrolysis pectin	
	Diseased	Uninoculated
Expt. 1		
Leaves	20.4	9.6
Petioles	17.3	8.2
Stems	12.7	8.0
Whole shoot	15.8	8.3
	Uninoculated	
	Diseased	Turgid Wilted
Expt. 2		
Whole shoot	23.2	11.4 20.2

When CMC was used for estimating cellulase activity, there was some evidence in the first experiment that extracts from diseased plants did cause a reduction of viscosity which was not caused by extracts from uninoculated plants, but the differences were small, and this result was not obtained in the second experiment.

As might be expected, the extracts had considerable pectinesterase activity. In the first experiment, 35 % of the total activity in the shoots from diseased plants was in the water extract; the corresponding figure for uninoculated plants was 21 %.

Dialysed extracts had about 60% of the activity of undialysed extracts. The results of the two experiments are summarized in Table 6 in which the figures are the percentage hydrolysis of methyl ester groups in 25 ml. 1% pectin solution in 1 hr. at pH 6.5 by a volume of extract corresponding to 1 g. of the fresh material.

In the first experiment the results could be interpreted only in terms of fresh weight and, on this basis, extracts from leaflets, petioles and the main stems of diseased plants were about twice as active as were those from uninoculated plants. Similar results for the whole shoot were obtained in the second experiment but this time activities could also be expressed in terms of dry weight and on this basis, if the activity of extracts from uninoculated turgid plants is 100, extracts from diseased and wilted plants had activities of 149 and 111 respectively. The tissues of diseased plants therefore contain substantially more pectinesterase than do tissues of healthy plants.

An attempt was also made to determine the pectinesterase activity of the roots of diseased and uninoculated plants but the results were unsatisfactory, primarily because the method of inoculation severed many of the roots, and because some of the roots grew into the bed of sand supporting the pots. It was very difficult, therefore, to know what proportion of the total root system was recovered. But in the second experiment, and on a fresh-weight basis, with the activity of shoot extracts as 100, extracts from roots of diseased, uninoculated turgid, and wilted plants had activities of 133, 252 and 210. And if the activity of healthy roots is 100, extracts from diseased and wilted plants had activities of 105 and 146 respectively. These figures would remain almost the same on a dry-weight basis because the roots of the three types of plants contained almost the same proportions of dry matter.

Enzyme activity of sap from healthy and diseased plants

Although large quantities of sap could be collected from some of the uninoculated plants, very little came out of the cut ends of any of the diseased plants. But sufficient was collected to test for viscosity-reducing enzymes and pectinesterase. Sap from diseased plants behaved no differently from that from healthy plants when tested for ability to reduce the viscosity of solutions of pectin, polypectate and CMC, but slight differences were obtained in the tests for pectinesterase in which 4 ml. of a 1% pectin solution containing 0.5 ml. sap and coloured lightly with bromo-thymol blue at pH 6.5 was incubated at 22°C. for 6 hr. under toluene. Over this period the acid liberated by the action of the different saps was as follows: Loran Blood, 0.25 ml.; Bonny Best healthy, 0.60 ml.; and Bonny Best diseased, 1.05 ml. of a 0.001 N solution.

Action of leaves and stems on enzyme

Five g. (fresh weight) laminae of leaflets from 8-week-old Bonny Best plants were ground finely in a mortar and added to 10 ml. water; 5 g. of sections of the main stem were treated similarly. One hour after the suspensions had been made, 10 ml. of filtrate from a culture grown on polypectate and casamino acids was added to each of the extracts, and to 10 ml. of water. Toluene was added and the mixtures were kept at 22°C. for 2 days. They were then centrifuged at 10,000 r.p.m. and the supernatant

solutions were collected, brought to the same volume, and tested on polypectate solutions at pH 5.5. The final pH values of the solutions were: leaf, 5.7; stem, 6.2; water, 8.1. The residues of leaf and stem after centrifuging were extracted in 10 ml. 0.5 M-NaCl at pH 8.0 for 15 hr. at 4° C. under toluene, and the extracts were collected and tested as before. The results are shown in Table 7.

Table 7. *Effect of leaves and stems on pectic enzymes*

Activity of filtrate incubated with water	100
Activity of filtrate incubated with leaf tissue	61
Activity of filtrate incubated with stem tissue	38
Activity of NaCl extract of leaf tissue	18
Activity of NaCl extract of stem tissue	24

About 39% of the activity was lost after incubation with leaf tissue, and 62% after treatment with stem tissue. Some of the loss could be attributed to adsorption of the enzyme on the tissue, but there was a loss of activity additional to this amounting to 21% for leaf tissue and 38% for stem tissue.

Extracts from suspensions incubated for 2 days without the enzyme, boiled for 10 min., and added to unboiled extracts before mixing with the polypectate had little effect on enzyme activity so it seems that there were no thermostable inhibitors present in the extracts and that the inactivation of the enzyme which was obtained was a relatively slow process.

Effect of culture filtrates and other solutions on cut shoots

(a) General effects

The general procedure was to take cuttings of Bonny Best plants with the terminal shoot and two or three expanded leaves and to put them in water for about 2 hr. The bottom 2-3 mm. of the stems of fully turgid cuttings were removed under water and the ends of the cuttings were then placed in the test solution contained in tubes or flasks on a laboratory bench at a temperature of 20-22° C. and about 4 ft. from two 80 W. fluorescent tubes. The pH of all solutions was adjusted to 6.0 before testing. A variety of symptoms appeared under different conditions, and some were unnatural in the sense that they would not normally be found in diseased plants. The most dramatic symptom was a collapse of the whole cutting, which hung at the side of the container and which then gradually dried out; inoculated plants never behaved in this way even when conditions were particularly favourable for disease development. More usually in these tests the main stem of the cutting below the level of the top shoot remained turgid and upright while the leaves wilted from below upwards until the top shoot and the uppermost, succulent part of the main stem wilted too. The leaflets wilted before the petioles of the leaves. Occasionally, necrotic spots, bluish black in colour, appeared in the leaflets, generally between the veins, and when this happened, the leaflets almost always remained more or less turgid. This was another symptom not seen in inoculated plants. In some experiments, chlorotic patches appeared on the leaflets, followed by desiccation of the tips and edges, symptoms very characteristic

of diseased plants. These symptoms appeared after cuttings had been in certain solutions for relatively long periods, 4 days or longer, and in cuttings which had wilted little if at all. Occasionally they were seen in plants which had wilted badly for some time but had recovered. Vascular discoloration was often found in cuttings, but it was generally atypical in the sense that the xylem became bluish black in colour and not brown as is more typical of diseased plants. In some solutions the ends of the cuttings became macerated and when this happened sections of the stem taken some distance from the cut end showed that the parenchyma around the vascular tissue was also disintegrated. If the stems of these cuttings were cut some 5-7 cm. from the base and gently squeezed the parenchyma around the vascular tissue oozed out over the surface, and when the pressure was released holes were left in the cortex immediately outside the vascular cylinder. This is another symptom which the writer has not found in inoculated plants.

(b) *Action of culture filtrates.* A large proportion of the filtrates obtained from cultures used to study the effect of substrate on enzyme production were tested on cuttings. Space does not permit giving the results in detail so only the more important findings will be given.

(1) Cultures grown on glucose or wood cellulose generally had much less effect than had cultures grown on other substrates, although in one series, wood cellulose filtrates caused far more extensive vascular discoloration than did the others; the vascular bundles were badly discoloured for 8-10 cm. from the base of the cuttings, whereas generally discoloration was confined to the bottom 1-2 cm.

(2) Cultures grown on pectin, polypectate or CMC caused the cuttings to wilt to varying extents. Occasionally there was a complete collapse of the shoot, but usually the main stem remained upright although the leaves wilted and became desiccated, the severity of these symptoms gradually increasing with time. Intervascular necrosis was an occasional symptom, obtained only with polypectate filtrates. When it occurred the petioles remained turgid and the cuttings took up water almost as quickly as did the controls. In general, the most natural and severe symptoms were obtained with pectin filtrates, although these were obtained from cultures which had grown far less than those on polypectate or CMC.

(3) Most culture filtrates caused some vascular discoloration but this rarely extended more than 2 cm. up the stem. When discoloration was most pronounced, wilting was always less severe, and the cuttings took up almost as much water as the controls.

(4) None of the filtrates had particularly high cellulase and pectinesterase activity, and the most active filtrates rarely caused the most severe symptoms. And although it was generally true that filtrates which most rapidly degraded polypectate and pectin also produced symptoms in cuttings most readily, there were some exceptions. One series of highly active filtrates caused only slight symptoms to develop after cuttings had been in them for over 24 hr. Tests of the filtrates after this period showed that little of the enzyme activity had been lost.

(5) Autoclaving filtrates only slightly reduced their wilting activity, but always reduced and sometimes eliminated the ability to cause vascular discoloration.

(6) Wilted cuttings regained their turgidity and normal appearance if the bottom 2 cm. were removed and the freshly cut ends were placed in water, provided the cuttings had not been in the filtrates for much over 24 hr. Sometimes they became turgid after they had been wilted for longer periods, but then the tips and edges of the leaflets remained, or became, shrivelled and desiccated.

(7) The above observations refer to filtrates from cultures grown on media with relatively high concentrations of carbon and nitrogen. Tests were also made with cultures grown in media based on xylem sap and 'synthetic sap'. Filtrates from cultures grown on sap with no additions had no effect on cuttings. Other filtrates, from media containing initially 0.2 % pectin, polypectate or CMC, caused the leaflets to become flaccid after 48 hours but none wilted badly; similar effects were caused by autoclaved extracts.

Filtrates from cultures grown on 'synthetic sap' media with added carbon substrates produced more severe symptoms. After 2 days in the pectin filtrates, the lower leaves were chlorotic, and the tips and edges of the leaflets were shrivelled and becoming desiccated, but the leaves did not wilt; vascular discoloration extended about 3 cm. up the stem. Similar symptoms were caused by the polypectate and CMC filtrates and, surprisingly, by the wood cellulose filtrates. After autoclaving the filtrates caused little or no vascular browning, and the other symptoms were much less severe although they were not eliminated.

(8) If in the experiments reported above more or less striking symptoms had not appeared in 24 hr. the cuttings were discarded, and it was assumed that the filtrates were inactive and of no interest. It was found, at least with some filtrates, that this assumption was wrong as the following will show. One series of filtrates, from cultures grown with 1 % different carbon substrates and 0.4 % casamino acids, were particularly active in degrading pectin and polypectate. They were far less active on CMC and had little pectinesterase activity. Surprisingly, these filtrates did not cause very striking symptoms after 24 hr. But two of them, from cultures grown on pectin and CMC, were used in other experiments in which attempts were made partially to purify the pectic enzymes by adsorption on Celite 800. The original filtrates, and solutions derived from them, were then tested on cuttings over 5 days. Each cutting was placed in 10 ml. of the test solution, and as the solutions were absorbed by the cuttings they were replaced by water. As in earlier experiments, no marked symptoms had appeared after 24 hr., but they appeared afterwards and were well developed after 4 days. Both culture filtrates caused similar effects so they will be described together.

Untreated filtrates had been diluted by a half before they were used; they caused extensive maceration of the bottom 2 cm. of the stem and disintegrated the parenchyma around the xylem elements some 10 cm. up the stem. Nevertheless, at no time did any part of the shoot symptom show more than transient wilting. But other symptoms appeared which were almost indistinguishable from those seen in badly diseased plants, the leaflets became chlorotic in patches and the tips and edges first of all shrivelled and later became desiccated. There was pronounced vascular browning for 9 cm. up the stem (2 cm. beyond the first node), and in the petiole of the first leaf.

There was no vascular browning in the lower 2 cm. of the stem which was badly macerated.

Untreated filtrates which had been diluted 10 times caused much the same effects on the leaves and upper part of the stem, but maceration was confined to the bottom 5 mm. and vascular discoloration did not extend more than 2.0 cm. up the stem.

Shoots were also treated with the following solutions: (i) Culture filtrate, diluted by a half, dialysed at 4° C. for 15 hr., and in a fresh lot of water for another 6 hr. (ii) Culture filtrate diluted by a half, 25 mg. Celite 800 added to 50 ml., stirred for 15 min., centrifuged and supernatant used. (iii) Celite from (ii) suspended in 0.2M phosphate at pH 8.0 for 15 min., centrifuged, and supernatant dialysed as in (i).

(i) and (ii) had about 3/20 activity of the undiluted culture filtrate on polypectate at pH 5.5; (iii) was almost inactive.

(i) and (ii) had much the same effect on shoots as had the untreated filtrates except that there was less maceration of the base, and vascular discoloration was limited to the lower 1-1.5 cm. Both disintegrated the parenchyma around the vascular bundles at some distance (3-5 cm.) from the base.

Symptoms on shoots treated with (iii) were far less pronounced and were confined to a slight chlorosis on one or two leaflets of the bottom leaf.

Portions of each of the above solutions were also used after they had been autoclaved for 15 min. at 15 p.s.i. Precipitates were produced which did not dissolve on standing: they were removed by centrifuging before the solutions were used.

Although some symptoms were produced by autoclaved solutions they were far less pronounced than those caused by the corresponding untreated solutions and none caused more than a trace of vascular discoloration.

Similar results were obtained when the experiment was repeated with a different lot of shoots, except that in cuttings treated with the most active solutions, the lowermost leaf wilted after 24 hr. and remained wilted subsequently.

In both experiments therefore, some of the enzymes which macerated the base of the cutting were translocated as much as 10 cm. through the xylem and passed out of the vessels to disintegrate the parenchyma of the vascular bundles. In spite of this there was very little wilting of the shoots, which took up water at a rate not much less than that of the controls. But with prolonged treatment symptoms characteristic of naturally diseased plants did appear, and might also have appeared in some of the earlier experiments had they been continued.

(c) *Solutions of degraded pectic substances.* It has been suggested that the action of cell-wall degrading enzymes produced by wilt fungi in xylem elements may lead to the release of compounds of relatively large molecular weight which would impede the flow of the vascular sap and so cause some of the symptoms found in diseased plants because it is known that a number of polysaccharides can induce wilting in tomato plants (Hodgson, Peterson & Riker, 1949). But so far as this suggestion relates to pectic substances and celluloses, it ignores the possibility that the action of the enzyme which releases large molecular weight compounds may continue until low molecular weight compounds are produced, and that these compounds would be far less significant. Some simple experiments illustrate this point.

Solutions of pectin, polypectate or CMC were degraded under toluene with active culture filtrates at 20–22° C. The enzyme/substrate mixtures consisted of 0.75 % pectin or polypectate, or 0.5 % CMC, 5 % untreated or autoclaved cell-free culture filtrate, HCl or NaOH to pH 6.0, M/40 phosphate buffer at pH 6.0. The culture filtrates were selected for their high activity; they caused a 50 % loss of viscosity of the polypectate pectin, and CMC solutions in about 2, 4 and 6 min. 15 hr. later the toluene was removed under vacuum, a series of dilutions of the mixtures were prepared and cuttings were placed into these solutions.

The results, summarized in Table 8, are shown as the lowest concentration of substrate (p.p.m.) which caused the shoots to wilt after different times. All concentrations above the one shown also caused shoots to wilt, whereas lower concentrations did not.

Table 8. *Effect of degraded substances on shoots. Lowest concentration (p.p.m.) causing wilting*

Substrate	1.5 hr.		3.0 hr.		5.0 hr.		24 hr.	
	Leaves	Shoots	Leaves	Shoots	Leaves	Shoots	Leaves	Shoots
Untreated pectin	468	468	234	468	234	468	29(b)	29
Degraded pectin	> 3750(a)	> 3750	> 3750	> 3750	> 3750	> 3750	1875	3750
Untreated polypectate	117	117	58	58	29	29	29(b)	29
Degraded polypectate	937	937	468	937	468	937	468	468
Untreated CMC	1250	2500	625	1250	312	625	78	78
Degraded CMC	> 2500(a)	> 2500	> 2500	> 2500	> 2500	> 2500	> 2500	> 2500

(a) Highest, and (b) lowest concentrations used.

After 24 hr. the bottom 2 in. of each stem were removed and the freshly cut end was placed in water; all shoots became turgid within 6 hr.

No precise figures were available, but on the basis of viscosity measurements the molecular weights of the untreated pectin, polypectate and CMC were of the same order so it was surprising to find that these substances differed so considerably in their ability to cause wilting. Polypectate was by far the most efficient in this respect and in the low concentrations of about 29 p.p.m. had caused the shoots to wilt in 5 hr. About ten times this concentration of pectin was needed to produce a similar effect. Nevertheless, pectin too was effective in quite low concentrations. In contrast, much higher concentrations of CMC were required for the same effect, at least in the relatively short period of 5 hr.

The action of the enzymes on pectin and CMC destroyed the ability of these substances to cause wilt even after 24 hr. at the highest concentration. Results with polypectate were less striking but here too enzyme action had reduced the wilting power of solutions by a factor of 1 to 10 or 20 in terms of concentration.

This experiment was repeated and similar results were obtained. Also, 24 hr. from the beginning the shoots were placed in a greenhouse and although at first more shoots wilted, there was gradual recovery so that after 3 days all shoots were turgid. But the leaves of those which had wilted most severely and for the longest period were

chlorotic with the tips and edge of the leaflets desiccated and shrivelled. No attempt had been made to keep the solutions sterile during this experiment and it was noticed that those containing autoclaved enzyme had lost most of their viscosity. This could have been caused by contaminants, or by traces of enzyme remaining after autoclaving.

DISCUSSION

When the isolate which was used in this work was grown on media relatively rich in nutrients, the culture filtrates rapidly degraded pectin and pectates in solution and macerated the tissues of tomato stems except for the vascular cylinder itself. This confirms work along similar lines by Kamal & Wood (1956), Talboys (1958), Scheffer *et al.* (1956). Surprisingly the cellulase activity, Cx *sensu* (Siu & Reese, 1953), of culture filtrates was relatively low, although the fungus grew about as well with carboxymethyl cellulose as with glucose as the main carbon source, and considerably better than on pectin or pectate. No direct evidence was obtained as to whether the fungus secreted cellulases able to degrade insoluble cellulose, C I (*sensu* Siu & Reese, 1953), but the fact that filtrates from cultures containing wood cellulose as the main carbon source did not act rapidly on solutions of carboxymethyl cellulose makes it improbable that C I type enzymes were produced in quantity. Nevertheless, the fact that the fungus did grow to some extent on wood cellulose must mean that C I enzymes were produced, although they did not accumulate in the filtrates.

Pectinesterase was present in some types of cultures, generally in those containing pectin, but most filtrates had a low activity or were virtually inactive.

Although the use of rich nutrient media can be justified when the main aims are to determine what types of enzyme a fungus *can* produce, and to obtain active preparations of particular enzymes, it must always be remembered that *in vivo* the pathogen probably never grows under these conditions. It was realistic, therefore, to attempt to grow the fungus in conditions less far removed from those obtaining in the plant. Fortunately this could be done because relatively large quantities of exudate can be obtained from the cut ends of tomato plants, and because it is probable that this exudate comes mainly from the xylem. The fungus grew very little in exudate when nothing was added, but did grow when various carbon sources were added. Glucose and pectate supported about the same growth, pectin and carboxymethyl cellulose gave somewhat less growth. Filtrates from cultures on glucose had little enzyme activity, but those grown on pectate and pectin rapidly reduced the viscosity of pectate solutions but were relatively inactive on pectin. Only cultures grown on carboxymethyl cellulose had any Cx activity and this was surprisingly high compared with the results obtained when rich media were used. There was very little pectinesterase activity in any of the filtrates.

From these results, therefore, it seems that chain-splitting pectic and cellulolytic enzymes would be produced in xylem vessels provided that relatively small quantities of appropriate substrates were available; from what is known about the structure and composition of the xylem elements this is very likely to be the case.

When plants were examined for pectic and cellulolytic enzymes it was found, some-

what paradoxically, that the only enzyme present in greater concentrations in diseased plants was pectinesterase which is not readily produced by the fungus in culture. Because of this, and because healthy tissue contains relatively large quantities of this enzyme, it is likely that the higher pectinesterase activity of diseased tissue is a response of the host and is not of fungal origin.

Because the methods for detecting the activity of chain-splitting enzymes are so sensitive under properly controlled conditions, the failure to demonstrate these enzymes in diseased plants means that they are not secreted, that they are secreted in small quantities which do not accumulate, or that they are inactivated soon after they are secreted. The first of these possibilities would seem unlikely in view of the results obtained with xylem exudates. The second is a possible explanation for the cellulases in view of the relatively low Cx activity of filtrates from cultures in which the substrate carboxymethyl cellulose had been used extensively for growth. There is some slight evidence for the third in that the pectic enzyme activity of filtrates was reduced when they were kept for some time in the presence of disintegrated tissue of tomato stems. This last aspect needs further study in view of recent work which has shown that pectic enzymes may be inactivated by phenolic and related substances (Byrde, 1957; Cole, 1958; Bell & Etchells, 1958) and the fact that vascular browning indicative of reactions involving phenols, is a constant feature of vascular wilt diseases.

If it be assumed that chain-splitting, pectic and cellulolytic enzymes are produced by the pathogen in the xylem, the question arises how, if at all, these enzymes cause disease symptoms. In this connexion there are a number of contradictory results to be reconciled. First, there can be little doubt that in diseased plants the flow of the vascular sap is greatly restricted, that some of the disease symptoms are characteristic of chronic water shortage in the leaves (Threlfall, 1959), and that the reduced flow is partly attributable to some sort of blockage of the xylem elements. Secondly, treatment of cuttings with partially purified preparations with pectic enzyme activity high enough to macerate the ends of the stem and the tissue around the bundles some distance up the stem, did not always cause the leaves to wilt although certain other symptoms were produced. Thirdly, degradation of carboxymethyl cellulose, pectin and pectates in solution greatly reduced the ability of these substances to cause wilting in cuttings, and pectates cause plants to wilt at much lower concentrations than pectin or carboxymethyl cellulose.

These facts can be reconciled, at least in part, by assuming that some of the pectic and cellulolytic enzymes produced by the fungus are adsorbed on the internal surface of the xylem elements, act for some time and release sufficient quantities of pectic and cellulose substrates in degraded form to permit continuous growth of the pathogen and continuous secretion of relatively small amounts of the enzymes. Erosion of the cell wall by these enzymes, and, possibly, enzymes acting upon other components, would release substances which the fungus either is unable to degrade or does so only to a very limited extent. These would accumulate in the lumen and impede the flow of the vascular sap. Accumulations of amorphous material are common in the vessels of diseased plants (Bewley, 1922), but the nature of this material is not known. If it is pectic or cellulosic in nature, it is difficult to see why it should not be degraded by

enzymes produced by the pathogen unless these are rapidly and preferentially adsorbed by the cell wall so that they are not available for degrading substances in the lumen of the vessels.

Degradation of the walls of the xylem elements, although it may be on a limited scale, may also release phenolic substances which could be acted upon by phenolases of the fungus or the host; products formed by the oxidation of the phenols may then be responsible for a continuous slow inactivation of pectic enzymes, and others, which are adsorbed on the cell walls.

It is also possible that not all of the pectic and cellulolytic enzymes are adsorbed locally on the cell walls but that some may be carried in the vascular stream and ultimately reach the parenchyma of the leaves, and damage them in some way. This had been suggested for the toxins believed to be secreted in tomato plants by *Fusarium oxysporum lycopersici*, and there would seem to be no reason why pectic enzymes should not act in a similar way. Solutions containing these enzymes kill cells of some tissues rapidly (Tribe, 1955), and very small quantities indeed would be required to produce some of the desiccation and necrosis which are often more characteristic symptoms of wilt diseases than wilting of the leaves and shoots.

In view of the circumstantial evidence which has been given above, and elsewhere (Talboys, 1958; Scheffer *et al.* 1956), further work on the pectin and cellulolytic enzymes of *Verticillium albo-atrum* as agents responsible for some symptoms of disease is justified. The main need now is for purified and well-characterized enzyme preparations and studies of their effects on the structure and physiology of tomato plants.

This work was done while the author was a temporary member of the staff of the Connecticut Agricultural Experiment Station. The author is greatly indebted to the Director, Dr J. G. Horsfall, for making this possible, and to Dr A. E. Dimond, head of the Plant Pathology Section, for his encouragement and unfailing kindness during the course of the work, and, later, for his advice about the manuscript. The author would also like to express his best thanks to the many other members of the Station staff, particularly Miss Barbara Wooding, who helped him in ways too numerous to mention.

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Potato haulm resistance to *Phytophthora infestans*

I. Field assessment of resistance

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(Received 1 June 1960)

SUMMARY

The resistance to potato blight (*Phytophthora infestans* (Mont.) de Bary) of the haulms of four potato varieties, Up to Date (UD), King Edward (KE), Majestic (MJ) and Arran Viking (AV) was assessed in the field from 1956 to 1959. Although these varieties are reputed to differ appreciably in resistance, the differences measured were smaller than expected and significant in only two seasons. They were shown better by measurements on individual leaves than by assessing effects on whole plots. The greater resistance of AV and MJ, compared with UD and KE, was correlated with a slower increase of blight at the start of an attack rather than with differences in the rate of haulm destruction when the attack was well advanced. However, there was some evidence that leaves of KE died most rapidly and leaves of AV most slowly after infection.

INTRODUCTION

The search for resistance to potato blight (*Phytophthora infestans* (Mont.) de Bary), and attempts to introduce resistance into commercial varieties, have continued for over a hundred years. Two types of resistance are distinguished: (1) field immunity, hypersensitive, racial or 'true' resistance (Müller, 1953) and (2) field resistance (Black & Gallegly, 1957).

The former was derived from clones of a wild Mexican species *Solanum demissum*, which were at first thought to be immune, but later were found to be hypersensitive, with host cells dying rapidly around infection sites. This reaction is controlled by a few major genes, the *R* genes (Black, 1952), and the transfer of at least one of them to hybrids with *S. tuberosum* was the basis for breeding hypersensitive varieties. The early promise of obtaining immunity to the disease (reviewed by Large (1940), Rudorf, Schaper, Ross, Baerecke & Torka (1950) and Black (1954)) was not fulfilled, because when such varieties were grown on a large scale they were soon attacked by new races of the fungus.

In 1954, Niederhauser, Cervantes & Servin reported that no clones of *S. demissum* were free from the disease in Mexico, but that a greater range of variability of the fungus could be expected there, because the sexual stage occurs in the field (Gallegly & Galindo, 1957), whereas it is unknown elsewhere and variation is considered to arise

only asexually (Toxopeus, 1956). Breeders realize that with the wide distribution of many of the known fungal races and the possibility of further races appearing, hypersensitivity based on single or multiple *R* genes is an inadequate protection against the disease.

Field resistance, variously called tolerance, multigenic, polygenic or partial resistance (Niederhauser *et al.* 1954) is complex, controlled by many genes and, unlike hypersensitivity, it seems to be effective against all known races of the fungus. Varieties are said to show field resistance when they are destroyed more slowly (stay green longer) in an epidemic or the disease causes less damage than in more susceptible varieties. Old-established varieties such as Champion were known to resist blight; between 1910 and 1919 Pethybridge was testing varieties which could withstand Irish epidemics, but either because of late maturity or poor commercial qualities they were less attractive to growers than existing blight-susceptible varieties. Following the discovery of hypersensitive resistance and the possibility of blight 'immune' varieties, most investigators neglected field resistance, but now it is again receiving attention. Already resistant varieties are beginning to replace more susceptible ones, e.g. Ackersegen and Voran are replacing the susceptible Coharila varieties in Chile and the resistant Dutch variety Robijn is replacing Kerr's Pink in Kenya (Cox & Large, 1960).

Early workers, e.g. Jones, Giddings & Lutman (1912), although knowing that varieties differed in their resistance to potato blight, often failed to detect in laboratory tests the resistance seen in the field, and the term 'field' resistance was introduced (Gallegly & Niederhauser, 1959). The term is used widely in plant pathology, but potato breeders have given it a particular meaning in relation to blight to include all forms of resistance except hypersensitivity (field immunity) (Black & Gallegly, 1957). Throughout these papers 'resistance' will imply multigene or field resistance, and hypersensitive resistance will be denoted by name.

The aim of the present investigation was to establish by field observation and laboratory experiments the differences between a few varieties, preferably of similar maturity, without *R* genes, but differing as much as possible in their haulm resistance to blight, and to devise methods whereby such resistance could be detected and assessed in laboratory tests, by techniques useful to plant breeders.

This paper deals with the assessment of the resistance of varieties in the field; later papers will deal with factors affecting infection of foliage and with the destruction of foliage by the fungus.

MATERIALS AND METHODS

Two widely grown maincrop varieties reputed to differ appreciably in blight resistance, the susceptible King Edward and moderately resistant Majestic, were used, together with a susceptible late variety, Up to Date, and Arran Viking, a maincrop which was described as having resistance more than or equal to that of Majestic. Blight resistance of these four varieties, referred to as the standard varieties, was assessed for the four years 1956 to 1959. In the 1959 experiment three more resistant varieties, as selected from observation plots in 1958, were also included in the field trial: Ackersegen (ex Germany), Ås (Norway) and Ontario (U.S.A.).

In all experiments with the British varieties 'A' certificate seed was used, whereas the foreign varieties were supplied by the National Institute of Agricultural Botany (Cambridge) from stock grown in Ireland.

Plots in field trials were usually arranged in a 4×4 Latin square design, but in 1959 four randomized blocks, each with seven varietal plots, were used. The detail of layouts will be described with the appropriate experiments. The rate of haulm defoliation was assessed by the British Mycological Society (BMS) key for blight destruction (Anon., 1947), where a quick over-all visual estimate is made of the percentage of the total haulm that has been killed by blight; and also more critically by frequently observing five tagged leaves on each of ten stems, indicated by canes, per plot. The leaves were selected at ridge height, some within the crop canopy and some exposed, and were marked with differently coloured polyvinyl chloride (P.V.C.) wire. Infection and subsequent destruction by blight or other causes was recorded on five leaflets per leaf, the terminal and the two distal pairs of leaflets. The frequency of observations depended on the speed and stage of the epidemic but they were made at least once per week. By observing the 1000 (5 leaflets per leaf $\times$ 5 leaves per stem $\times$ 10 stems per plot $\times$ 4 replicate plots) leaflets per variety defoliation could be related to infection, which cannot be done with the BMS key. Tagged leaves were found to be invaluable for studying differences between varieties, and observations made on them will be referred to throughout this work.

EXPERIMENTAL RESULTS

Field experiment 1956

Plots, six rows, each 28 inches apart, by 27 feet, of the varieties Up to Date, King Edward, Majestic and Arran Viking were set out in a 4×4 Latin square, and each was surrounded by a guard area of Majestic. Blight was first seen in the experimental area on 23 July, and waves of infections, after favourable weather, rapidly defoliated the plants, all of which were destroyed by the second week in September.

Weekly assessment of haulm destruction by blight using the BMS key was difficult because of (a) the different growth habits of the varieties, (b) the small size of the plots, and (c) the subjective nature of the key. Nevertheless, it was clear that King Edward was defoliated more rapidly than Up to Date, with no difference detectable between Arran Viking and Majestic, but both were more resistant than Up to Date, especially during the early stages of the epidemic (Fig. 1).

The use of the tagged-leaf method, which is less subject to personal error, showed larger differences between the varieties than did the use of the BMS key. The numbers of leaflets destroyed on each occasion were expressed as percentages of the total marked, correcting for losses through other causes and are plotted against time in Fig. 1. When resistance was assessed as a 'prolongation of growth' at half-decay (Large, 1945) (50% destruction), the tagged leaves showed a difference of 6 days between the extreme varieties King Edward and Arran Viking, and of 2 and 4 days between King Edward and Up to Date and Majestic respectively. Using the BMS key, there was a difference of only 3 days between the King Edward and Majestic, and Arran Viking appeared no

more resistant than Majestic. Analysing the transformed percentage destruction of the tagged leaves for each occasion showed significant differences between the varieties (Table 1). Leaflets were destroyed less rapidly in Arran Viking than in Up to Date and King Edward throughout, and less rapidly than Majestic during part of the epidemic. Majestic also differed significantly from King Edward.

Large (1952) showed that a well-timed spraying with fungicide prolonged the initial lag phase of the blight attack and that, after the effect of the spray had disappeared,

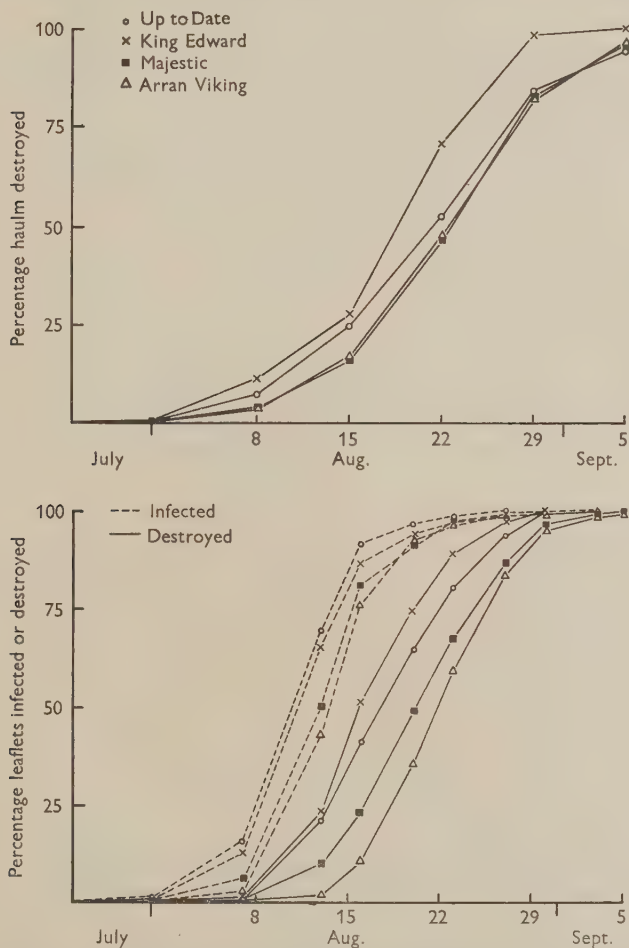


Fig. 1. Effect of blight on standard varieties 1956. Upper graph: assessment of haulm destruction using BMS key. Lower graph: tagged-leaf method recording percentage leaflets infected and percentage destroyed.

Table 1. *Rate of destruction of tagged leaflets of the standard varieties in the 1956 blight epidemic*

(% leaflets destroyed.)

Variety	Date of observation								
	25. vii.	31. vii.	7. viii.	13. viii.	16. viii.	20. viii.	23. viii.	27. viii.	30. viii.
Up to Date	0	0	1.4	21.5	40.9	64.7	80.5	93.4	99.4
	—	—	—	26.7	39.6	53.5	64.1	—	—
King Edward	0	0.8	1.8	23.9	51.3	74.6	89.2	97.2	99.5
	—	—	—	26.7	46.0	60.5	73.5	—	—
Majestic	0	0.2	0.4	10.2	23.3	49.2	67.5	86.7	96.5
	—	—	—	16.7	27.7	44.6	55.5	—	—
Arran Viking	0	0.1	0.3	2.9	10.9	35.8	59.4	83.7	95.1
	—	—	—	7.8	17.8	36.8	50.5	—	—
LSD ($P = 0.05$)	—	—	—	12.9	8.1	6.6	6.54	—	—

Analyses done on angular transformations which are in bold type.

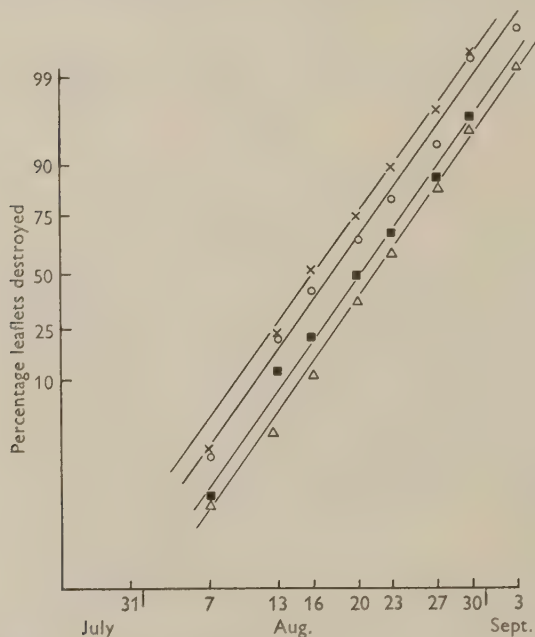


Fig. 2. Assessment of haulm destruction 1956. Percentage tagged leaflets destroyed plotted on a probit scale. ○, Up to Date; ×, King Edward; ■, Majestic; △, Arran Viking.

haulm destruction followed a course almost parallel to that of the unsprayed control. The differences in resistance shown by Majestic and Arran Viking compared with Up to Date and King Edward are somewhat analogous to the effects of spraying,

for after an initial delay the course of leaflet destruction almost parallels that of the more susceptible varieties. This suggests that the 'resistant' varieties have haulms intrinsically as susceptible as those of King Edward and Up to Date, but that some factor limits their infection during the early stages of the epidemic (Fig. 2). I shall show in a later paper that fewer spores are produced on the 'resistant' than on the susceptible varieties, so a limiting factor may be lack of inoculum.

Field experiment 1957

The plots in 1956 were surrounded by a crop of Majestic, and it was thought this might explain the unexpectedly small difference in resistance shown by Arran Viking: the plots were small and might have been influenced by the Majestic, especially if amount of inoculum is important. To overcome this defect, two experiments were done in 1957. In one, the plot area was increased to twenty rows by 46 feet but observations made only in a central area of eight rows by 18 feet. This design also made possible a study of guard areas for edge effects to assess interaction between varietal plots.

The other experiment was of the same total dimensions, but the potato observation areas of eight rows by 18 feet were surrounded by a blight-immune crop, kale (cv. Cansón Thousand Head).

Adverse weather prevented the potato-in-kale experiment from being planted until 2 weeks later than the all-potato experiment, and this delay caused a difference in the development of the two crops, which persisted throughout the season. Blight appeared in both experiments at the beginning of August and developed rapidly at first, especially in the all-potato one, but later the weather became drier and defoliation continued slowly until the end of September.

Both experiments were useless for assessing differences in resistance between varieties because the incidence of blight differed greatly between replicate plots and obscured any varietal differences, which were never significant (see Discussion). In the potato-in-kale experiment, King Edward and Up to Date were most susceptible but in the all-potato area King Edward was, unexpectedly, the least affected by blight.

The study of edge effects made in the all-potato experiment, by comparing the haulm destruction, using the BMS key, of the guard areas with that of the central observation area of plots, failed to show a significant interaction between susceptible and more resistant varieties.

Field experiment 1958

Because of the conflicting results obtained in 1956 and 1957, the experiment to assess resistance to blight was repeated in 1958. The plots, ten rows by 23 feet, were surrounded by blight-immune crops. Eight rows of kale were sown between plots within columns of the Latin square design and the areas 28 feet long between plots within columns were planted with mangolds on the ridges to ease potato cultivations.

Blight was first found on 18 July in one King Edward plot, and all plots of all varieties were then infected artificially by inoculating five leaves, to ensure that the fungus was present in all in an attempt to avoid the uneven distribution encountered

in 1957 (see Discussion). The weather was continually wet and all the potato plants were defoliated by the end of August.

In assessing progress of disease, the results from the tagged leaves were treated as in 1956. Plotting the percentages of infected leaflets against time (Fig. 3) shows there was a difference of 4 days between the extreme varieties at 50% defoliation. Angular transformation shows that the differences between Arran Viking and Majestic were not significant, but that these varieties differed significantly from Up to Date and King Edward. As in 1956, probits show a very similar rate of destruction in all four varieties. The initial rate of infection of tagged leaves (Fig. 3) was delayed in Majestic and Arran

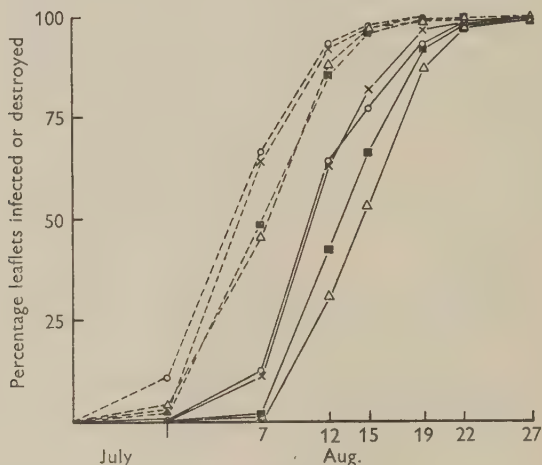


Fig. 3. Effect of blight on standard varieties 1958, using tagged-leaf method, recording percentage leaflets infected and percentage destroyed. O, Up to Date; x, King Edward; ■, Majestic; Δ, Arran Viking; ---, infected; —, destroyed.

Viking, as in 1956; however, a similar lag in King Edward did not result in slower destruction than in Up to Date. By contrast, although infection was as rapid in Arran Viking as in Majestic, destruction was slower.

Field experiment 1959

The small differences between the four varieties used so far prompted a search for more resistant ones. In small field plots in 1958, three varieties, Ackersegen, Ås and Ontario, showed more resistance than the others, and were included in the 1959 experiment, done in four randomized blocks each of seven varietal plots. Plots were surrounded by guard areas of kale and mangolds as in 1958.

The season was dry and plants grew poorly. Ontario, grown from cut seed, produced so few plants that it had to be excluded from the experiment. Only Up to Date and Ackersegen formed a dense canopy; the other varieties remained open until storms caused stems to straggle across the furrows.

Blight was first seen on 12 August, when all plots were infected artificially as in 1958. Spread continued, mainly following storms, until the end of August and by the end of September most of the haulm was dead except of Ackersegen, which was still green when lifted in the third week of October. The results from tagged leaves (Fig. 4) show that, although there were no new infections after the end of August, there were already enough eventually to destroy about 85 % of the leaflets in Up to Date, King Edward, Majestic and Arran Viking, 63 % in Ås and 33 % in Ackersegen. This destruction was caused by the fungus passing from infected leaflets into leaf stalks and stems. King Edward was most heavily infected by blight and was defoliated most rapidly,

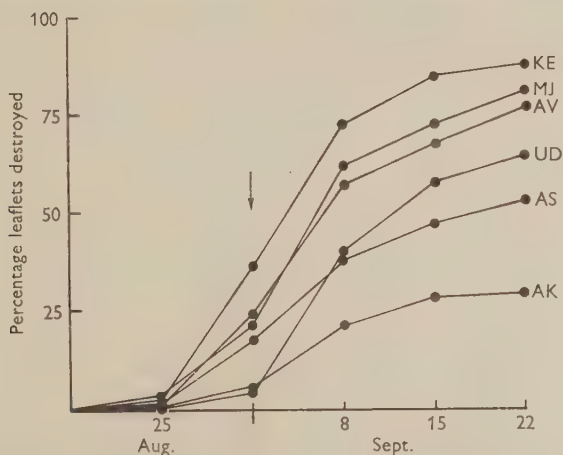


Fig. 4. Assessment of haulm destruction 1959 using tagged-leaf method. KE (King Edward), MJ (Majestic), AV (Arran Viking), UD (Up to Date), AS (Ås) and AK (Ackersegen). ↓, no new infections of leaflets recorded after this date.

whereas Up to Date was defoliated more slowly than in previous years. Ås and Ackersegen were least affected, confirming the 1958 observation of their higher level of blight resistance in comparison with that of the standard varieties.

DISCUSSION

The differences in blight resistance between the four standard varieties when compared in the field, although significant, are smaller than expected from commercial experience. Nevertheless, they could be important, because prolonging the growth of the haulm by one week during July or August may represent an increase in yield of a ton or more per acre (Doncaster & Gregory, 1948). The different lengths of time taken by the fungus to destroy these varieties did not depend primarily on different rates of haulm destruction during the epidemic phase but more on an initial delay at the start of the blight attack. This delay could be attributed in the resistant Majestic and Arran Viking to a slower rate of infection of leaflets, and although this also

occurred on King Edward in 1958 (Fig. 3), it did not retard the rate of destruction, which was as rapid as in Up to Date. When the difference in time between 50% tagged leaflets infected and 50% destroyed is calculated for each season (Table 2), King

Table 2. *Intervals (days) between 50% leaflets infected and 50% destroyed in the standard varieties*

	Up to Date	King Edward	Majestic	Arran Viking
1956	6.6	4.8	6.5	8.2
1957 { (a) potato	5.5	1.7	5.0	5.1
(b) in kale	3.2	2.2	4.8	5.8
1958	5.6	5.2	5.8	7.0
Mean	5.2	3.5	5.5	6.5

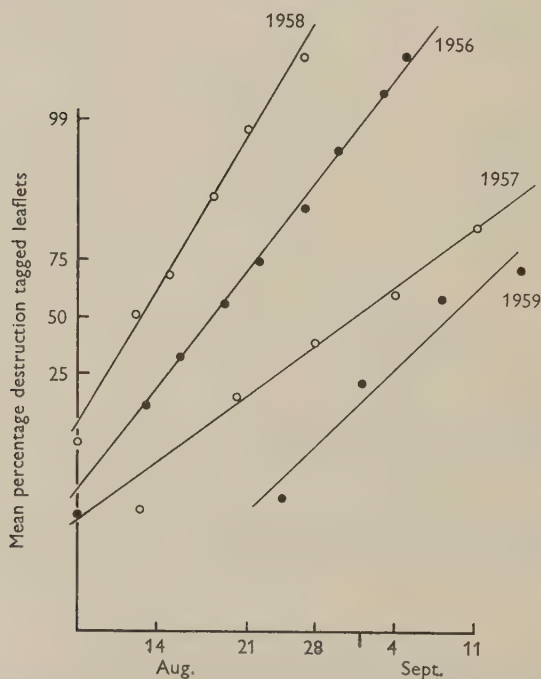


Fig. 5. Seasonal variation in the progress of blight epidemics (means of the four standard varieties are plotted on a probit scale). Data for 1957 calculated from the all-potato experiment only.

Edward leaves are found to be destroyed most rapidly after infection. In Arran Viking, generally the most resistant of the standard varieties, leaflets are destroyed the least rapidly after infection. The results suggest that the resistance factors which retard the early development of the blight attack in the 'resistant' varieties, are

insufficient later to prevent the disease from becoming epidemic, or to slow down the rate of defoliation.

The tagged-leaf method, although laborious, proved useful because it allowed a detailed study to be made of the progress of the disease. Records could be kept of time of infection, the number and position of lesions on leaves and leaflets, and the amount of destruction. Although the complete destruction of leaflets was the criterion used in plotting graphs, records were also made of partially destroyed leaflets, assessing the

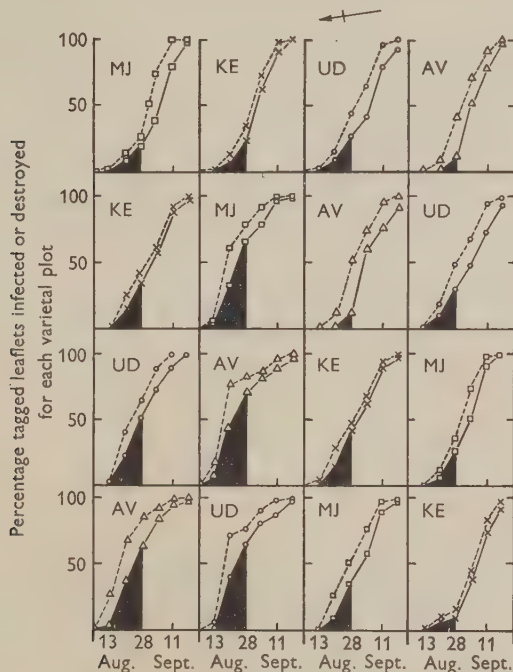


Fig. 6. Assessment of haulm destruction 1957. All-potato experiment. Percentage of tagged leaflets infected (dotted line) and percentage destroyed (continuous line) calculated for each varietal plot. Symbols: ○, Up to Date; ×, King Edward; □, Majestic; and △, Arran Viking. Shaded area indicates destruction on 28 August.

affected areas by eighths. The conclusions remain the same, however, whether the partially destroyed leaflets are included or not.

The disadvantage of the tagged-leaf method is the loss of leaves from other causes, such as wind damage, *Botrytis cinerea* (which after being saprophytic on fallen flowers, parasitizes healthy leaves) and senescence, especially of leaves in the lower canopy. The loss from senescence depends on when leaves are tagged in relation to the outbreak of the disease and on the rate of progress of the epidemic, which varies from

year to year (Fig. 5). The rule has been not to tag leaves until blight is present in the plots.

The failure of field experiments in 1957 to show significant differences between the resistance of varieties can be attributed to several factors; the randomization, the time of infection of plots and the slow progress of the epidemic (Fig. 5). The infection and destruction curves plotted for the plots separately in the all-potato experiment (Fig. 6) show that blight developed most rapidly in the north-west corner, affecting two plots of Up to Date and Arran Viking whereas in the south-east not only was the crop development much poorer but there was also much less infection, which again affected two plots of these varieties. This uneven disease distribution caused large plot-to-plot errors which were not accounted for when analysed by row or column effects, because King Edward and Majestic were not affected in the same way. Surprisingly the disease developed slowest on King Edward, which in the final analysis appeared most resistant. A similar analysis of the potato-in-kale experiment again showed an uneven distribution of blight throughout the varieties with poor replication between plots. In 1956, although one column of plots was infected artificially to study spread around an infected plant and allowances were made for this in the treatment

Table 3. *Percentage tubers of standard varieties infected with blight at lifting*

	Up to Date	King Edward	Majestic	Arran Viking
1956	11.0	9.3	1.4	1.4
1957 { (a) potato	17.4	11.1	1.3	1.8
(b) in kale	29.4	19.2	3.8	1.9
1958	12.0	4.7	0.8	0.3
Mean	17.4	11.1	1.8	1.3

of results, distribution of the disease was more uniform with good replication between plots. Artificially introducing the disease into all plots, as in 1958 and 1959, when the first natural infections were found, may have minimized the differences between varieties, but even natural infection may arrive in experiments sooner than in commercial practice because of the proximity of susceptible and resistant varieties.

Epidemics may sometimes start from a primary focus of infection which originates from an infected shoot produced by a diseased tuber (Hirst, 1955) and hence the tuber susceptibility of a variety must be considered as well as that of the haulm (van der Zaag, 1959). When the percentage of blighted tubers recorded at lifting (Table 3) is considered for the standard varieties, more are found infected on Up to Date and King Edward than on Majestic and Arran Viking and, unlike estimates of haulm resistance, differences were consistent from season to season. Considering the number of blighted tubers alone, there is a greater chance of a primary focus originating from the tuber of Up to Date and King Edward; however, the likelihood of blight overwintering will depend, not only on the numbers of tubers infected during the epidemic, but also on the resistance of tubers to rotting and more research is required into both of these aspects. If crops of resistant varieties need to be infected from neighbouring

susceptible varieties, this delay in infection will tend to accentuate their innate haulm resistance and perhaps, in commercial practice, this is true for Majestic and Arran Viking.

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Saprophytic colonization of wheat straw by *Cercospora herpotrichoides* Fron and other fungi

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(Received 1 April 1960)

SUMMARY

In liquid cultures *Cercospora herpotrichoides* utilized both organic and inorganic sources of nitrogen and showed an absolute requirement for thiamin. On nutrient agar and through sterilized straw, *C. herpotrichoides* grew more slowly than other facultative fungal parasites of cereal roots and stem-bases.

The competitive saprophytic ability of *C. herpotrichoides* was found to be very limited. The observation that saprophytic colonization of straw tissue by *Ophiobolus graminis* declined as invasion progressed inwards was confirmed and found to occur also with *C. herpotrichoides* and *Helminthosporium sativum* but not with *Fusarium culmorum*.

It is concluded that *C. herpotrichoides* exhibits a low competitive saprophytic ability and is therefore unlikely to colonize straw in the soil.

INTRODUCTION

Information concerning the saprophytic activity of facultative parasites in soil, as opposed to their behaviour in pure culture, is limited. Garrett (1950) reviewed the position with regard to the root-inhabiting fungi, and Butler (1953*a, b, c*), Lucas (1955) and Rao (1959) reported on the competitive saprophytic ability of *Curvularia ramosa*, *Helminthosporium sativum*, *Fusarium culmorum* and *Ophiobolus graminis*.

The present paper extends these investigations to *Cercospora herpotrichoides* Fron., the fungus responsible for the eyespot disease of cereals and which attacks the lower leaf-sheaths and stem-bases of the plants, eventually causing lodging. Macer (1955, 1961) showed that this fungus survived in infested tissue in the soil during the intervals between susceptible host crops but its precise status during this period is not known. Comparisons of saprophytic ability have, therefore, been made between *C. herpotrichoides* and the vigorous competitive saprophyte *F. culmorum* which is a regular colonizer of dead and senescent wheat straw (Sadasivan, 1939), and between *C. herpotrichoides* and *O. graminis* which can only colonize wheat straw to a limited extent in competition with the soil microflora (Lucas, 1955).

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EXPERIMENTS UPON THE NUTRITIONAL REQUIREMENTS OF
*CERCOSPORELLA HERPOTRICHOIDES**Isolation and culture of Cercospora herpotrichoides*

Isolates of *C. herpotrichoides* were obtained from the stem-bases of mature wheat, barley and oat plants showing characteristic eyespot lesions. Air-dry stems were split open and the mycelium found within the lumen was plated-out on 2% potato-dextrose agar (P.D.A.). On this medium the mycelium slowly developed as a velvety grey colony which frequently became raised in the centre although considerable variations in both colour and habit were noted between isolates.

Except where otherwise stated, a single isolate of *C. herpotrichoides* (A. 14) was used for all the experiments. The cultures were stored on P.D.A. slopes under oil at +3° C. and tested frequently for pathogenicity which appeared to remain unchanged during the four years that the cultures were under observation.

Utilization of nitrogen sources

As the experiments on competitive saprophytic colonization by *C. herpotrichoides* were planned to incorporate nutritional amendments to the substrate (sterilized mature wheat straw), the nutritional and growth factor requirements of the isolates of the fungus had first to be determined. Blair (1954) showed that peptone was the most suitable source of nitrogen in sand culture and that nitrate nitrogen was not utilized at a low pH. He found also that the growth factors biotin, pyridoxin, nicotinic acid and aneurin (thiamin) did not produce growth in a casein hydrolysate medium equal to that on a peptone medium alone.

In the present experiments the fungus was grown as an unshaken liquid culture in 180 ml. acid-washed medicine bottles. The basal medium used throughout contained *d*-glucose (2%), KH_2PO_4 (0.4%), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2%), CaCl_2 (0.04%), NaCl (0.04%) and a minor element mixture similar to that described by Beadle & Tatum (1945).*

In the first experiment growth of *C. herpotrichoides* with nitrate nitrogen (as sodium nitrate), as measured by increase in mycelial dry weight, was compared with that with ammonium nitrogen (as ammonium tartrate) and with nitrogen contained in two organic sources, hydrolysed casein and peptone. These sources were compared at three concentrations, 0.01, 0.02 and 0.04% nitrogen which, with 2% *d*-glucose in the basal solution gave, respectively, carbon:nitrogen ratios of 20, 40 and 80. Thiamin HCl and biotin were added to the basal medium at standard rates of 0.1 and 0.03 mg./l.

Three bottles in each nutritional series were inoculated with 4 mm. agar discs taken from the margin of a colony growing on pyridine-washed water agar. After 25 days' incubation at 22.5° C. the mycelial pads from the three replicates were pooled, washed and dried at 75° C. to constant weight. No significant difference was found between the dry weights of the pooled samples, thus demonstrating that this isolate of

* $\text{Na}_2\text{B}_4\text{O}_7 \cdot \text{H}_2\text{O}$, 0.01 mg.; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1 mg.; $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$, 0.2 mg.; $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$, 0.2 mg.; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.02 mg.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 2.0 mg./l. of basal medium.

C. herpotrichoides could utilize all four sources of nitrogen at the concentrations tested. A similar experiment using ten further isolates of *C. herpotrichoides*, obtained from a range of host species, showed that all the isolates were able to utilize nitrate nitrogen but the differences in dry weights obtained between isolates indicated that after 37 days' incubation at 22.5° C. considerable variations in growth rate occurred (Table 1).

Table 1. *Nitrate nitrogen utilization by ten isolates of Cercospora herpotrichoides in culture*

Isolate no.	Origin	Host	Mycelial dry weights (mg.)*	
			0.02 % N†	Control
52-IIIc	Norfolk	Wheat (Yeoman)	802	14
52-Vc	Surrey	Wheat (Bersee)	613	14
52-VIc	Sussex	Wheat (Hybrid 46)	587	10
52-VIIc	Sussex	Wheat (Cappelle-Desprez)	1012	17
52-VIIIc	Sussex	Wheat (Glasnevin Rosa)	848	16
52-Bc	Isle of Ely	Barley (Plumage-Archer)	987	19
52-Oc	Cambridge	Oat (S. 147)	547	12
E 10	New Zealand	<i>Agropyron</i> sp.	711	10
E 12	New Zealand	Wheat (unknown)	821	15
E 14	New Zealand	<i>Phleum</i> sp.	1084	20

* Total of three replicates.

† Nitrogen supplied as sodium nitrate.

Growth factor requirements

Using a similar technique the effect of omitting thiamin and biotin, singly and together, from the basal medium was studied. Four sources of nitrogen were again used but at only a single concentration. The pooled mycelial dry weights recorded after 25 days' incubation at 22.5° C. (Table 2) show that significantly increased

Table 2. *Biotin and thiamin requirements of Cercospora herpotrichoides in culture*

0.02 % nitrogen supplied as	Mycelial dry weights (mg.)*			
	Control, no growth factors	Biotin	Thiamin	Biotin and thiamin
Nitrate	10	31	334	247
Tartrate	22	38	217	203
Casein	35	78	282	222
Peptone	44	71	267	286

* Total of three replicates.

Least significant difference between growth factor means for $P = 0.05$, 57.8 mg.; $P = 0.01$, 87.5 mg.; $P = 0.001 = 140.6$ mg. (S.E. = ± 23.6).

mycelial dry weights were obtained throughout by adding thiamin. Biotin increased growth slightly in the absence of thiamin: in the presence of thiamin, however, biotin tended to depress growth. In both the casein hydrolysate and peptone series in the

absence of thiamin the dry weights were approximately double those for the sodium nitrate and ammonium tartrate series but this was probably due to traces of thiamin later detected as an impurity in the casein hydrolysate and peptone.

None of the other growth factors investigated, viz. riboflavin (0.1 mg. l.), nicotinic acid (0.1 mg. l.), pyridoxin (0.05 mg. l.), inositol (0.4 mg. l.), *p*-aminobenzoic acid (0.1 mg. l.), pantothenic acid (0.2 mg. l.) and adenine (0.1 mg. l.) produced a significant increase in dry weight over the controls. The only combination of growth factors to out-yield the same substances supplied singly was that of biotin and pyridoxin. It may be concluded that all the isolates of *C. herpotrichoides* used in these experiments were unable to synthesize thiamin, a condition which is frequently found in pathogenic fungi.

EXPERIMENTS UPON GROWTH RATE

C. herpotrichoides is frequently difficult to isolate from wheat stems clearly showing eyespot lesions because of the rapid invasion of the diseased tissue by secondary parasites and obligate saprophytes, such as *F. culmorum* (W. G. Sm.) Sacc. and *F. avenaceum* (Fr.) Sacc., which rapidly overgrow *C. herpotrichoides* on agar. If the poor performance of *C. herpotrichoides* on isolation plates could be demonstrated to be due to its inherently slow growth, and not to unfavourable incubation conditions or nutritional deficiencies in the agar medium, then this could be an important factor in limiting the competitive saprophytic ability of the fungus.

Linear growth-rate over an agar surface

The linear growth-rate of *C. herpotrichoides* on P.D.A. was compared with that of four other fungi parasitizing the roots or stem-bases of cereal plants, namely *Helminthosporium sativum* Pammel, King & Bakke (*Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur), *Curvularia ramosa* (Bain.) Boedijn., *Ophiobolus graminis* (Sacc.) Sacc. and *Fusarium culmorum* (W. G. Sm.) Sacc.

Table 3. *Effect of temperature on linear growth-rate of Cercospora herpotrichoides and four other fungi*

Temp. (°C.)	Mean increase of colony diameter (mm./24 hr.)				
	<i>C. herpotrichoides</i>	<i>H. sativum</i>	<i>C. ramosa</i>	<i>O. graminis</i>	<i>F. culmorum</i>
27.0	0.3	12.3	13.0	13.2	21.6
25.0	1.1	11.3	11.3	19.3	24.0
22.5	2.5	7.6	9.6	16.0	21.5
16.0-20.0	2.5	7.0	6.3	12.1	18.0

Pilot colonies were first grown on P.D.A. and 4 mm. marginal discs were used to inoculate six replicate plates for each of the four temperature treatments. Linear growth-rate was recorded by measuring two diameters of the colonies, at right angles, at 24 hr. intervals.

Table 3 shows that the most rapid rate of linear growth at all temperatures was that of *F. culmorum*, a vigorous competitive saprophyte (Sadasivan, 1939; Butler, 1953*a*;

Rao, 1959), and the slowest growth was shown by *C. herpotrichoides*. The exceptionally slow growth of *C. herpotrichoides* compared with the other fungi studied could be a critical factor in restricting its competitive saprophytic colonizing ability. *C. herpotrichoides* also differs from the other fungi in having a lower optimal temperature for growth.

Growth through sterilized straw tissue

Because the rate of fungus growth over an agar surface does not necessarily indicate its rate of growth within natural substrates *C. herpotrichoides* and the four other fungi were cultured on sterilized straw and their rate of penetration of this tissue determined. This was assessed from the time taken by each fungus to grow through an autoclaved wheat stem, surrounded by its sheath, and subsequently to contaminate a piece of straw fitted within the lumen. Lengths of wheat straw (variety Wilma) were cut with a razor, 7.5 cm. above and 1.5 cm. below a node; those straws with an internal diameter outside the range 4.5–5.0 mm. at 7.5 cm. above the node and those with split stems were rejected. A 2.5 cm. length of culm straw was inserted into the lumen,

Table 4. *Penetration of wheat straw by Cercospora herpotrichoides and four other fungi*

(Number of straws penetrated out of 45 tested.)

Fungus	Number of hours' incubation at 22.5° C.														
	36	48	60	72	84	96	108	120	132	144	156	168	180	192	
<i>C. herpotrichoides</i>	0	0	0	0	0	0	0	0	10	21	29	37	45	45	
<i>H. sativum</i>	0	0	19	27	45	45	—	—	—	—	—	—	—	—	
<i>C. ramosa</i>	0	9	14	37	45	45	—	—	—	—	—	—	—	—	
<i>O. graminis</i>	0	0	0	0	0	4	28	45	45	—	—	—	—	—	
<i>F. culmorum</i>	0	0	12	30	45	45	—	—	—	—	—	—	—	—	

pushed down to the nodal plate and held in position by a cotton-wool plug. To prevent colonization of the inserted straw by hyphae entering through orifices in the nodal plate, the lumen of the straw below the node was filled with 'plaster of paris'; the cut upper end was plugged with cotton-wool. The straws were then soaked, steamed and autoclaved.

Inoculum of each fungus was grown on maize-meal-sand culture medium (100 parts dry sand, 3 parts maize-meal and 13 parts water) and after 21 days 150 g. was transferred aseptically to a sterilized covered glass tumbler containing 120 g. saturated sand. Ten of the sterilized straws were pressed into each partially filled tumbler and dry sterilized sand added to cover the inoculum to a depth of 1 cm. The tumblers were incubated at 22.5° C. and at intervals of 12 hr. random samples of fifteen straws for each fungus were taken and the inserted pieces of straw withdrawn aseptically and tested for the presence of the inoculant fungus by incubation on P.D.A. plates. The results obtained from three replicates for each fungus are given in Table 4 and show that all the fungi penetrated wheat straw much more slowly than they grew over an agar surface. A comparison between Tables 3 and 4 further shows that the relative rates of penetration of straw cannot be predicted with certainty from measurements of

growth on agar. The comparatively rapid penetration of straw by *F. culmorum* reflects its status as a vigorous competitive saprophyte and post-harvest colonizer of cereal stubble. The slow penetration of straw by *O. graminis* and the even slower penetration by *C. herpotrichoides* suggest a very low competitive saprophytic ability for the latter fungus.

However, to confirm that a fungus is a competitive saprophyte in soil it is necessary to show that it can colonize the appropriate organic substrate in competition with the natural soil microflora.

EXPERIMENTS UPON SAPROPHYTIC COLONIZATION

The competitive saprophytic ability of *C. herpotrichoides* in comparison with that of *O. graminis* and *F. culmorum* was assessed by growing cultures of the test fungi upon maize meal-sand culture medium and then diluting this with increasing proportions of an unsterilized soil, thus progressively reducing the inoculum potential* of the inoculant fungus in relation to that of the natural soil microflora (Butler, 1953a; Lucas, 1955). A suitable number of wheat straws was then buried in each of the inoculum/soil dilution mixtures. The soil used (Kettering loam) was collected from a field dump immediately before use and passed through a 2 mm. sieve, after which the moisture-holding capacity (M.H.C.) was determined (Keen & Raczkowski, 1921).

The autoclaved 500 ml. flasks of maize meal-sand culture medium were inoculated with 6 mm. marginal disks from colonies on P.D.A. The flasks containing *C. herpotrichoides* were incubated for 42 days at 22.5° C. and those containing the other fungi were incubated for 28 days. The incubation periods differed because *C. herpotrichoides* grew much more slowly than the other fungi on this medium.

The maize meal-sand inoculum and soil were mixed, in proportion by dry weight, to give the dilution series 98:2, 90:10, 50:50, 10:90 and 2:98; soil and maize meal-sand inoculum were used as controls. All the inoculum/soil dilution mixtures were then adjusted to 45 % M.H.C. which was maintained throughout the experiments.

Pieces of straw were cut 0.5 cm. below and 2.5 cm. above a node and soaked under reduced pressure in solutions of 2 % *D*-glucose (D), 1 % sodium nitrate (N), 1 % sodium nitrate and 2 % *D*-glucose (D+N) and distilled water (O). The straws were then drained and autoclaved. In some experiments unsterilized straws (UO) were used, which were only soaked in distilled water. The straws from each pre-treatment were mixed in batches of fifty with 200 g. of the inoculum/soil dilution mixtures in glass containers, incubated for 28 days at room temperature and then examined as described below.

Assessment of saprophytic colonization of straw

(a) *Cercospora herpotrichoides*

Direct plating on agar was unsatisfactory for assessing the saprophytic colonization of straw by *C. herpotrichoides* because colonies of this fungus were rapidly suppressed by fast-growing saprophytes. A seedling test was used in some experiments but this

* Inoculum potential was defined by Garrett (1956, p. 41) as 'the energy of growth of a fungus available for colonization of a substrate at the surface of the substrate to be colonized'.

was superseded by a modification of the 'sand-plate' method (Butler, 1953*a*) in which the test straws were placed upon moist sterilized sand to promote sporulation.

Seedling test. In the seedling test fifty wheat seeds were germinated in trays partly filled with compost and a test piece of straw, after removal of the node, was slipped over each coleoptile. Sand was then sieved over the boxes to the level of the top of the straws which held them in position and also prevented drying out. After 8–12 weeks' growth in an unheated glasshouse the seedlings were harvested and examined for the presence of eyespot lesions (Plate). A disease rating was calculated from an estimate of the degree of infection on a 0–4 basis, but the results agreed closely with percentage infection scores and the latter only are recorded in this paper.

'Sand-plate' method. During the winter, between September and May, good sporulation of *C. herpotrichoides* was obtained on straws incubated for 21 days in Petri dishes of saturated sand ('sand-plates') in a plant shelter. From June until August it was necessary to remove the 'sand-plates' to a refrigerator at +1° C. from 9 a.m. until 6 p.m. to obtain the low and fluctuating temperatures required for sporulation (Glynne, 1953).

Positive identification of *C. herpotrichoides* was not possible by direct examination of the straw surface because the spores are small, hyaline and develop upon acervulae. Samples of spores were, therefore, scraped off the straw and examined. In this way the acicular, slightly curved, multiseptate, spores of *C. herpotrichoides* can be clearly distinguished from those of other fungal colonizers of wheat straw. The percentage of straws in each sample producing spores of *C. herpotrichoides* was recorded together with an estimate of intensity of sporulation (Macer, 1961). This modification of the 'sand-plate' method makes possible the detection in straw of fungi with spores which are difficult or impossible to identify by direct observation *in situ*.

(b) *Fusarium culmorum*

F. culmorum does not always produce specific disease symptoms upon wheat seedlings and was therefore assessed by the modified 'sand-plate' method.

(c) *Ophiobolus graminis*

O. graminis was assessed by the seedling test (Garrett, 1938) carried out by planting a wheat grain, germ end downwards, in the lumen of each straw piece, growing the seedlings in sterilized sand in trays and later examining the root systems for lesions, cortical discoloration and runner hyphae.

(d) *Helminthosporium sativum*

The presence in the straw of this fungus was detected by the original 'sand-plate' method as described by Butler (1953*a*).

EXPERIMENTAL RESULTS

(a) Colonization of nodal straw by *Cercospora herpotrichoides* and *Fusarium culmorum*

The colonization of nodal straw by *C. herpotrichoides* was investigated using five straw pre-treatments and five inoculum/soil dilution mixtures. Colonization was assessed after 28 days' incubation at room temperature (18–21°C.) by both the seedling and the 'sand-plate' methods. Each sample was based upon 100 straws and the results obtained are given in Table 5 which shows that colonization by *C. herpotrichoides* took place only in those inoculum/soil dilution mixtures containing a high proportion of inoculum. In general, the figures recorded by the 'sand-plate' method

Table 5. Colonization of nodal straw by *Cercospora herpotrichoides*. Seedling and 'sand-plate' methods

		Percentage straws colonized					
		D			UO		
		Seedling	Sand-plate	Mean	Seedling	Sand-plate	Mean
Inoculum	100 %	89	100	95	57	70	64
	98 %	42	62	52	8	12	10
	90 %	24	20	22	0	0	0
	50 %	0	0	0	0	0	0
	10 %	0	0	0	0	0	0
	2 %	0	0	0	0	0	0
Soil		0	0	0	0	0	0

Sterilized straw pre-treatments: D = 1 % *d*-glucose. Unsterilized straw pre-treatment: UO = distilled water.

Table 6. Colonization of nodal straw by *Fusarium culmorum*

		Percentage straws colonized					
		D	N	D+N	O	UO	Mean
Inoculum	100 %	100	100	100	100	64	93
	98 %	76	100	96	92	50	83
	90 %	66	100	100	100	79	90
	50 %	93	100	95	100	65	91
	10 %	100	100	100	100	56	91
	2 %	100	100	96	100	41	87
Soil		9	15	4	0	7	7
Mean		78	88	84	85	52	—

Sterilized straw pre-treatments: D = 1 % *d*-glucose, N = 1 % NaNO₃, D+N = 1 % *d*-glucose + 1 % NaNO₃, O = distilled water.

Unsterilized straw pre-treatment: UO = distilled water.

and the seedling test agree, but in the 90 % inoculum/soil mixture the colonization recorded by the seedling test was consistently higher. Possibly the sporulation in sparsely colonized tissue was reduced either by unfavourable conditions or by competition from other micro-organisms which did not affect hyphal growth to the same

extent. The various nutritional amendments to the sterilized straws did not increase colonization by *C. herpotrichoides*. Throughout the unsterilized straw series, colonization by *C. herpotrichoides* was markedly reduced, indicating the inability of this fungus to compete even with micro-organisms present in a dormant state when the experiment began.

A similar experiment with *F. culmorum* (see Table 6) showed that the wheat straw was colonized at all inoculum/soil dilutions tested and that a little colonization also occurred in the soil control series. In all treatments colonization of the unsterilized straw by *F. culmorum* was lower than that of the sterilized straw. In this experiment nitrate nitrogen did not have the depressant effect recorded by Lucas (1955) but this may have been due to the different criteria of colonization employed (viz. the ability to cause seedling infection compared with macrospore formation).

(b) Investigation of the 'leaf-sheath' effect

Lucas (1955) demonstrated that with *O. graminis* percentage colonization of inter-nodal straw (without a leaf-sheath) was greater than colonization of culm surrounded by its sheath. The leaf-sheath appeared, in fact, to be acting as a zone in which *O. graminis* was competing unsuccessfully with more vigorous saprophytes.

Table 7. Colonization of leaf-sheath and culm by *Helminthosporium sativum* after washing and brushing

		Percentage colonized					
		Leaf-sheath			Culm		
		D	O	Mean	D	O	Mean
Inoculum	100 %	94	100	97	100	100	100
	98 %	42	84	63	45	83	64
	90 %	65	88	77	54	74	64
	50 %	16	18	17	8	10	9
	10 %	0	0	0	0	0	0
	2 %	0	0	0	0	0	0
Soil		0	0	0	0	0	0

In the present investigation, this test was repeated using the weakly competitive saprophyte *H. sativum*. Four straw pre-treatments were used with five inoculum/soil dilution mixtures. The nodal straws, after 28 days' incubation in the inoculum/soil dilution mixtures, were surface-sterilized with mercuric chloride and washed. The node was cut off and the culm withdrawn from the leaf-sheath, culm and leaf-sheath were then placed separately on 'sand-plates' and examined after 21 days' incubation at 22.5° C.

The results indicated that the surface sterilizing agent had damaged some of the hyphae established within the leaf-sheath but that the effect was less marked upon the protected and more solid culm. The experiment was therefore repeated without surface sterilization, giving the results listed in Table 7.

These show a slightly higher level of colonization in the sheath than in the culm,

thus confirming the killing effect of the mercuric chloride in the leaf-sheath. Results of a similar experiment using *F. culmorum* as the inoculant fungus are recorded in Table 8. There is a generally lower level of colonization than that recorded in Table 6 possibly due to the removal, by more thorough washing, of mycelium aggregated between the leaf-sheath and the culm which contributed to the degree of sporulation shown by the nodal straws. The intensity of colonization of the leaf-sheath was less than that of the culm suggesting that a vigorous saprophyte such as *F. culmorum* might 'increase' its recorded colonization with continued progress through the straw; the reverse of the behaviour of the two weakly competitive fungi *O. graminis* and *H. sativum*. It is, however, also possible that the leaf-sheath may not be such a suitable substrate as the culm for saprophytic colonization by *F. culmorum*.

Table 8. Colonization of nodal straw by *Fusarium culmorum*. Leaf-sheath and culm incubated separately

		Percentage colonized					
		Leaf-sheath			Culm		
		D	O	Mean	D	O	Mean
Inoculum	100 %	56	62	59	84	84	84
	98 %	52	48	50	83	80	82
	90 %	43	38	41	78	74	76
	50 %	40	36	38	63	62	63
	10 %	40	38	39	70	60	65
	2 %	36	29	33	64	56	60
Soil		8	10	9	14	12	13

Table 9. Colonization of leaf-sheath, culm protected by leaf-sheath, and culm alone by *Cercospora herpotrichoides*

		Percentage colonized								
		D			O			UO		
		S	C ^s	C ^o	S	C ^s	C ^o	S	C ^s	C ^o
Inoculum	100 %	100	100	97	100	100	100	80	20	—
	98 %	88	75	56	100	55	78	32	12	—
	90 %	69	14	40	100	30	69	22	0	—
	50 %	51	14	32	70	27	69	0	0	—
	10 %	8	0	2	18	0	9	0	0	—
	2 %	0	0	0	0	0	5	0	0	—
Soil		0	0	0	0	0	0	0	0	—
Mean		45	29	32	55	30	47	19	5	—

S = Leaf-sheath; C^s = culm incubated within leaf-sheath; C^o = culm incubated without leaf-sheath.

A further experiment with *C. herpotrichoides* (see Table 9) compared colonization, in inoculum/soil dilution mixtures, of three straw types: (1) internodal culm incubated without a leaf-sheath (C^o), (2) nodal culm incubated within the leaf-sheath (C^s), and (3) the leaf-sheath itself (S). This procedure was necessary because a direct comparison of colonization in the leaf-sheath with that of the enclosed culm might be

misleading as differences other than those of position are involved, i.e. there are differences in both structure and composition between the leaf-sheath and culm.

This experiment was carried out at a lower temperature (16–19° C.) than the previous experiments and a generally higher level of colonization than that recorded in Table 6 was found. The distilled water pre-treatment induced the highest over-all level of colonization; the lowest colonization was again found in unsterilized straws. Comparing the three straw types, most colonization occurred in the leaf-sheath (S) and least in the culm incubated within the leaf-sheath (C^s). This suggested that the presence of the leaf-sheath reduced the colonization of the enclosed culm and this conclusion is supported by the higher degree of colonization in the unenclosed culm (C^o).

The results of these experiments indicated that the inoculum potential of *F. culmorum* slightly increased during the colonization of the leaf-sheath, whereas that of *C. herpotrichoides* was reduced. At the time of mixing the inoculum/soil dilutions with the straw pieces all fungi had an opportunity of entering the sterile straw surface in proportion to their inoculum potential. Weakly competitive saprophytes may, however, become well established in the outer layers of tissue if present, as in these experiments, in an active mycelial form as the inoculant fungus. Fungi of high competitive saprophytic ability would, however, penetrate more rapidly through the tissues and their inoculum potential would progressively increase relative to that of less vigorous saprophytes with continuing advance through the substrate.

Table 10. *Colonization of concentric layers of straw by Cercospora herpotrichoides, Ophiobolus graminis and Fusarium culmorum*

		Percentage straws colonized								
		<i>C. herpotrichoides</i>			<i>O. graminis</i>			<i>F. culmorum</i>		
		A	B	C	A	B	C	A	B	C
Inoculum	100 %	100	100	100	100	100	100	100	100	100
	98 %	88	12	0	90	83	52	79	70	80
	90 %	23	2	0	88	77	50	79	65	84
	50 %	0	0	0	69	28	6	78	90	87
	10 %	0	0	0	0	0	0	—	—	—
Soil		0	0	0	0	0	0	63	50	55
Mean		35	19	17	58	48	35	80	75	81

A = outer layer; B = central layer; C = inner layer (next to glass rod).

In a further experiment with *C. herpotrichoides*, *F. culmorum* and *O. graminis*, the zone of competition was extended from a single leaf-sheath to three concentric layers of 10 cm. lengths of straw tied around a 12 cm. glass tube. The straw bundles, after being soaked in distilled water, were wrapped in greaseproof paper and autoclaved in containers with 200 g. saturated sand covering the cut lower ends to delay the later entry of fungal hyphae. The containers were packed with inoculum/soil dilution mixtures and the paper covering of the straw bundles was removed immediately prior to incubation. After 28 days at 22.5° C. the bundles were removed, the straw layers

separated and the presence or absence of the inoculant fungus in the central 3 cm. of each straw determined as previously described. To carry out the seedling test with *O. graminis*, however, the lower end of each straw piece was blocked to ensure that the seedling roots thoroughly explored the straw tissue.

The data given in Table 10 show a progressive reduction in the percentage colonization from the outer to inner straw layers by both *C. herpotrichoides* and *O. graminis* in all the pre-treatment series. No such reduction was observed with *F. culmorum*. This experiment, which extended in both space and time the factors controlling the 'leaf-sheath effect', provides further evidence that the inoculum potential of weakly competitive saprophytes decreases centripetally in relation to that of the other constituents of the soil microflora, whereas that of the vigorous saprophyte was not reduced although the increased colonization previously noted (Table 8) was not observed in this experiment.

The very slow rate of growth of *C. herpotrichoides* on agar and through sterilized straw is probably the major factor determining the 'leaf-sheath effect' and the low percentage colonization of sterilized straws in the inoculum/soil dilution mixtures. The latter declined rapidly with increasing dilution of the inoculum and, as the rate of this decrease can be taken as an inverse measure of the saprophytic colonizing ability of a fungus, it appears that *C. herpotrichoides* has an extremely low competitive saprophytic colonizing ability. *C. herpotrichoides* may be even more restricted in this respect than *Ophiobolus graminis* and it seems unlikely, therefore, that *C. herpotrichoides* exists either as a free-living saprophyte or as an active competitor for plant debris in soil.

I am indebted to the Master and Fellows of St John's College, Cambridge, for the award of a Hutchinson Research Studentship during the tenure of which this work was begun, and to the Agricultural Research Council for a Research Studentship. I wish to thank Dr S. D. Garrett for much valuable advice and encouragement; Dr H. C. Smith for supplying the New Zealand isolates of *Cercospora herpotrichoides*, and Mr F. T. N. Elborn for the photograph.

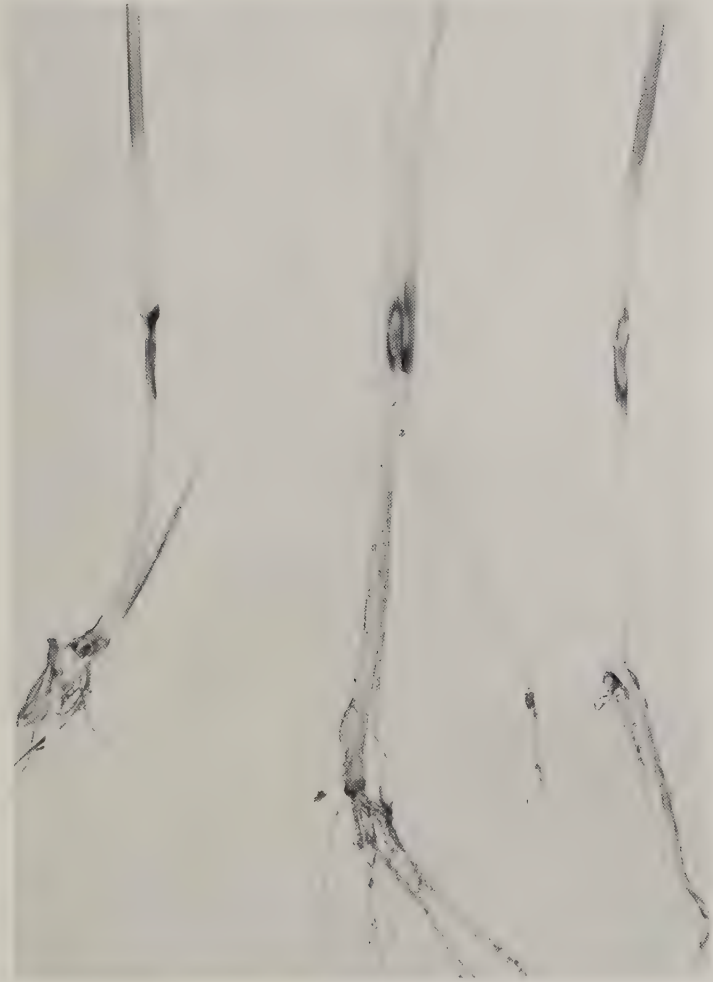
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EXPLANATION OF PLATE

Seedling test. Wheat seedlings showing characteristic eyespot lesions. Centre and right, leaf-sheath attached. Left, leaf-sheath removed.



The survival of *Cercospora herpotrichoides* Fron in wheat straw

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(Received 1 April 1960)

SUMMARY

The saprophytic survival of *Cercospora herpotrichoides* in naturally infected and artificially infested wheat straw has been investigated. Test pieces of straw containing the fungus were buried both in unsterilized soil in laboratory containers and in the field. Survival of the fungus was assessed by its ability to produce viable and infective spores on the surface of the recovered pieces of straw when incubated in a moist chamber. It was found that *C. herpotrichoides* survived, virtually undiminished in vitality, in straw buried for 3 years, the maximum period tested. Straw colonized by *C. herpotrichoides* was observed to decompose more slowly in the soil than did uninoculated straw; this suggests a mechanism whereby *C. herpotrichoides* may be enabled to survive for such long periods in the presence of competitors.

INTRODUCTION

The fungus *Cercospora herpotrichoides* Fron. is responsible for the eyespot foot-rot of cereals. Severe losses, particularly in wheat, are caused by this disease in Northern and Western European countries (Fron, 1912; Oort, 1936; Glynne, 1936), and the disease also occurs in North America (Sprague, 1931), South Africa (Van Der Watt & Nortje, 1953), New Zealand (Blair, 1954), and Australia (Butler, 1957). Eyespot is a soil-borne disease which cannot be controlled by short breaks from susceptible crops in the rotation, and Glynne (1942), Storey (1947) and Glynne & Moore (1949) have reported that breaks of 2 years or more did not completely control the disease. Macer (1961*b*) has found cases of eyespot on fields which have not carried a susceptible crop for 5 successive years, but under most conditions a break of 2-3 years from wheat or barley is sufficient to give practical control of the disease, although not always to eliminate the fungus.

Macer (1961*a*) has demonstrated that *C. herpotrichoides* is characterized by a low competitive saprophytic colonizing ability and that it is therefore unlikely to survive in soil as a free-living saprophyte. Blair (1954) was unable to isolate *C. herpotrichoides* from soil attached to, or directly beneath, diseased plants.

Precise information about the saprophytic survival of facultative parasites in infected tissue is available for very few fungi. Christensen (1922) reported that

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naturally infected wheat and barley straw left unprotected in the field contained viable mycelium of *Helminthosporium sativum* (*Cochliobolus sativus*) after 14 months. Katznelson (1940) was able to re-isolate this fungus 83 days after the inoculation of unsterilized soil with spores, but attempted re-isolation after 167 days was unsuccessful. Blair (1954) found that stubble, naturally infected with *C. herpotrichoides*, produced spores after 3 months' burial when placed under suitable conditions, and that straw remnants capable of sporulation occurred in fields in New Zealand 2 years after an infected crop had been harvested. Garrett (1938) working with *Ophiobolus graminis*, and Butler (1953) working with *O. graminis*, *H. sativum* and *Curvularia ramosa*, found that all these fungi survived in infested straw tissue for the maximum period tested in the laboratory (28–34 weeks), although the level of survival was affected by the nitrate nitrogen content of the soil.

The present paper describes experiments to investigate the saprophytic survival of *C. herpotrichoides* in wheat straw buried in soil.

METHODS

The techniques used were based upon those described by Garrett (1938, 1940) but the viability of *C. herpotrichoides* was assessed by the 'sand-plate' method (Butler, 1953; Macer, 1961*a*). In one experiment *O. graminis* was used for comparative purposes and in this case a seedling test was used to assess viability.

Because spore production by *C. herpotrichoides* on naturally infected stubble was found to be variable artificially infested pieces of straw had to be used in the later experiments. Sterilized nodal wheat straw (2.5 cm. lengths) was incubated in flasks containing a well-grown culture of the fungus on maize-meal-sand medium for 56 days at 22.5° C., at the end of which time 100% of the straw pieces gave a positive test for the presence of the fungus. The straws were then washed and buried in laboratory containers or in the field. In the laboratory experiments fifty straws were buried in each container (1 lb. glass jam-jars) holding the equivalent of 200 g. air-dry unsterilized soil maintained at 45% moisture holding capacity. The containers were incubated at room temperature (16–21° C.) and sampled at intervals in random pairs giving 100 straws. These were placed on saturated sand in Petri dishes ('sand-plates') and incubated for 21 days at high humidity and low fluctuating temperatures to induce sporulation (Glynne, 1953). Three spore samples were obtained from each straw by scraping with a scalpel and examined separately in a drop of water. The frequency of spores of *C. herpotrichoides* in each of five randomly selected microscope fields was coded as follows: 0 = no spores; 1 = 1–10; 2 = 11–50; 3 = 50–c. 200; 4 = > c. 200. A sporulation rating (SR) for each sample of straws was derived thus:

$$SR = \frac{100 \times (\text{individual ratings})}{4 \times (\text{number of straws examined})}$$

EXPERIMENTAL

Survival in soil in the laboratory

Experiment 1. Stubble showing characteristic eyespot lesions was cut into 2.5 cm. lengths and buried in soil in laboratory containers. Two soils were used: an organic loam (pH 4.8) from Kettering, low in potash but high in organic matter, and a clay loam (pH 8.1) from the Rockefeller Field Station of the Cambridge Botany School. Samples of straws were taken at 5-weekly intervals and the results (Table 1) are expressed as a percentage of straws developing spores of *C. herpotrichoides* and as a sporulation rating.

Table 1. *Survival of Cercospora herpotrichoides in naturally infected straw buried in different soil types in the laboratory*

Time from burial (weeks)	Soil type			
	Kettering		Field Station	
	(%)*	(SR)†	(%)	(SR)
0	68	40	68	40
5	74	42	66	38
10	60	36	70	40
15	62	32	66	30
25	56	34	60	32
30	54	28	60	24
35	56	30	52	28

* Percentage straws showing sporulation of *C. herpotrichoides*.

† Sporulation rating.

These data show that in naturally infected straw placed under optimal conditions for sporulation only 68 % produced spores at the outset of the experiment. Nevertheless, there was little reduction in percentage survival of *C. herpotrichoides* or in intensity of its sporulation after 35 weeks' burial, and there was no apparent difference in survival between the two soil types used.

Experiment 2. This experiment compared the longevity of *C. herpotrichoides* and of *O. graminis* in saprophytically colonized straw buried in soil in laboratory containers. *O. graminis* effectively colonized the straw after 28 days' incubation in the maize meal-sand inoculum, but *C. herpotrichoides* required a further 28 days' incubation before colonization was complete. The excess inoculum was removed and the straws buried in two batches of Kettering loam: in one batch sodium nitrate was added at the rate of 0.5 g. nitrogen per 100 g. dry weight straw; in the other batch nitrate nitrogen was reduced by pre-incubation with excess straw for 28 days under conditions encouraging maximum microbiological activity (Garrett, 1940). The nitrate nitrogen contents of the two batches at the beginning of the experiment were 26.3 and 0.4 mg./100 g. air-dry soil, respectively. Samples of 100 straws from each batch were taken at 4-weekly intervals and the results are shown in Table 2.

The saprophytically colonized straw proved more satisfactory than did naturally infected straw and the control straws showed 100 % sporulation of *C. herpotrichoides*.

Table 2. *Comparison of longevity of Cercospora herpotrichoides and Ophiobolus graminis in artificially infested straws buried in unsterilized soil in the laboratory*

Time from burial (weeks)	Survival in nitrate-enriched soil			Survival in nitrate-impoverished soil		
	<i>O. graminis</i> (%)	<i>C. herpotrichoides</i>		<i>O. graminis</i> (%)	<i>C. herpotrichoides</i>	
		(%)	(SR)		(%)	(SR)
0	100	100	76	100	100	76
2	100	100	71	100	100	63
4	100	84	51	100	95	68
8	100	100	70	94	98	82
12	100	100	70	72	100	55
16	98	100	72	48	92	60
20	86	95	66	26	86	51
26	63	60	35	4	54	28
38	10	60	30	0	48	20

After 38 weeks' burial the viability of *O. graminis* was reduced to 10% in the nitrate-enriched soil and to nil in the nitrate-impoverished soil, thus confirming the findings of Garrett (1938). *C. herpotrichoides* showed in general a higher level of survival but the effect of available nitrogen was less marked than with *O. graminis*, although there is some indication of a similar trend.

Survival in the field

Experiment 3. The previous experiment has shown that in the laboratory, where microbiological experiments are much accelerated by a high soil temperature, *C. herpotrichoides* survived at a high level for the maximum period tested. Survival in the field was investigated at the Rockefeller Field Station of the Cambridge Botany School, where, in September 1953, an experiment was laid down on land which had carried a wheat crop during summer and part of which had been undersown with a clover-ryegrass mixture. Artificially infested internodal pieces of straw were placed on the soil surface and others were buried in nylon nets in separate trenches at depths of 2.5, 15 and 45 cm. in two series; one under stubble and one under the ley. The stubble plots were kept weed-free throughout the experiment and the ley plots were cut three times each year. At intervals up to 156 weeks 100 test and uninoculated control straws from each trench, and from the soil surface, were examined for spores of *C. herpotrichoides*. Sporulation started on the surface straws in both fallow and ley series within 4 weeks, continued all through the winter and ended on the stubble-fallow in April and on the ley in May. A little sporulation re-started in the following October, but fewer spores were found on the samples taken from the fallow than on those from the ley. No spores of *C. herpotrichoides* were found on the buried straws at the time of lifting nor on any of the controls. However, after this preliminary examination the straws were washed and incubated on 'sand-plates' for 21 days and again examined for spores of *C. herpotrichoides*.

The results given in Tables 3 and 4 show that after 156 weeks' (3 years) burial a high percentage of straws recovered from depths of 15 and 45 cm. produced large numbers

of spores when placed under suitable conditions. Straws from the surface series sporulated heavily up to the 34th week but thereafter sporulation fell steeply and ceased after the 79th week. At a depth of 2.5 cm. percentage survival began to decline after the 79th week and was much reduced by the end of the experiment. On the straws from the 15 and 45 cm. levels the fungus showed a 70% survival after 3 years. No consistent differences were observed in survival in buried straws between the fallow and the ley series. Spores of *C. herpotrichoides* were not found on the uninoculated straws.

Table 3. *Survival of Cercospora herpotrichoides in artificially infested straws buried under a clover-ryegrass ley*

Time from burial (weeks)	Uninoculated control (all depths) (%)	Depth of burial (cm.)							
		0 (surface)		2.5		15.0		45.0	
		(%)	SR	(%)	SR	(%)	SR	(%)	SR
0	0	100	100	100	100	100	100	100	100
4	0	20	10	56	39	84	65	83	68
12	0	—	—	80	56	87	75	92	84
28	0	100	93	100	97	100	97	100	98
34	0	84	46	98	76	100	78	95	79
56	0	12	5	96	81	100	90	100	93
62	0	11	3	92	74	100	95	100	89
79	0	14	10	92	76	100	86	—	—
120	0	0	0	37	9	89	35	76	40
156	0	0	0	23	7	76	40	70	38

Table 4. *Survival of Cercospora herpotrichoides in artificially infested straws buried under fallow*

Time from burial (weeks)	Uninoculated control (all depths) (%)	Depth of burial (cm.)							
		0 (surface)		2.5		15.0		45.0	
		(%)	SR	(%)	SR	(%)	SR	(%)	SR
0	0	100	100	100	100	100	100	100	100
4	0	58	40	62	48	63	40	80	68
12	0	—	—	92	80	96	82	100	87
28	0	88	64	92	86	100	97	100	96
34	0	100	86	92	84	96	81	100	69
56	0	8	2	74	43	100	64	96	72
62	0	8	3	84	57	93	66	100	69
79	0	5	2	60	32	—	—	—	—
120	0	0	0	30	10	88	48	67	44
156	0	0	0	18	6	76	40	70	38

Spores from all the straw samples showed high viability on water agar. Spores from straws buried for more than 56 weeks were tested for infectivity by spraying on to wheat seedlings of the variety Yeoman grown in a cold glasshouse. In all tests typical eyespot lesions developed 6 weeks after inoculation.

*Observations on rate of decomposition of straw infested by
Cercospora herpotrichoides*

Buried straws infested with *C. herpotrichoides* decomposed more slowly than did the control uninoculated straws and, when placed on 'sand-plates', they developed a less varied fungal microflora. Straws showing abundant spores of *C. herpotrichoides* produced few spores of other fungi but the control straws developed fructifications and spores of many fungi, e.g. perithecia of *Chaetomium* spp. and macrospores of *Fusarium* spp. This suggested an antagonistic effect of *C. herpotrichoides* towards other straw-colonizing fungi, thus delaying the decomposition of cellulose and lignin.

Experiment 4. To compare the rates of decomposition in soil in the laboratory of straw inoculated with *C. herpotrichoides* and of uninoculated straw 2.5 cm. lengths of internodal straw were soaked in water and sterilized in flasks. One half of the flasks was inoculated with a maize-meal-sand culture of *C. herpotrichoides* and incubated at 22.5° C. for 28 days. The infested and control straws were then washed out and the equivalent of 2.5 g. dry weight of each (at 75° C.) buried in soil in laboratory containers. Two soils were used differing in nitrate nitrogen content as described in Expt. 1. After 4, 8 and 16 weeks respectively two containers were selected at random from each series and the straws washed, dried at 75° C. and weighed.

Table 5 shows that both infested and uninoculated straws lost weight more rapidly in the nitrate-enriched series than in the nitrate-impoverished series and that the uninoculated straws decreased in dry weight more rapidly than those inoculated with *C. herpotrichoides*.

Table 5. *Decomposition of uninoculated straw and straw inoculated with
Cercospora herpotrichoides in soil at two nitrate levels*

Time (weeks)	(Dry weight straw (g.))			
	Nitrate-enriched soil		Nitrate-impoverished soil	
	Uninoculated	Inoculated	Uninoculated	Inoculated
0	5.00	5.00	5.00	5.00
4	3.92	4.14	4.02	4.82
8	3.11	3.36	3.35	3.53
16	2.34	2.63	3.01	3.33

Experiment 5. To test the ability of one isolate of *C. herpotrichoides* to utilize a soluble cellulose compound (sodium carboxymethylcellulose—'Cellofas B') as a carbon source the fungus was grown in three media: (a) 1% 'Cellofas B', (b) 1% *d*-glucose, and (c) no added carbon source. In this experiment the basal medium contained 0.02% nitrogen (as sodium nitrate), 0.4% potassium dihydrogen phosphate, 0.02% crystalline magnesium sulphate, 0.04% calcium chloride, 0.04% sodium chloride, thiamin HCl (0.1 mg. per litre) and trace elements. The pooled mycelial dry weights obtained from the three replicates in the experiment are given in Table 6.

Since 'Cellofas B', which is more readily available than natural cellulose to most micro-organisms that have been tested, is shown by Table 6 to be such a poor carbon

source for *C. herpotrichoides*, it seems unlikely that this fungus can decompose natural cellulose to any significant extent. This result, and the fact that all straws after incubation with *C. herpotrichoides* gave a strong positive response to a test for lignin, indicates that this fungus is unlikely to destroy mature straw rapidly.

Table 6. Utilization of 'Cellofas B' and d-glucose by *Cercospora herpotrichoides*

(Mycelial dry weights (mg.))		
No carbon source	1 % 'Cellofas B'	1 % d-glucose
22	74	363

C. herpotrichoides might, however, modify the establishment of other straw-colonizing fungi by the production of an antibiotic substance. Three isolates were examined, but no anti-bacterial or anti-fungal activity was found after 24 days' growth on malt extract medium although after 38 days slight anti-fungal activity was detected (Brian, 1954). It is doubtful whether this can explain the observations recorded above, and it is therefore tentatively suggested that *C. herpotrichoides*, once established in straw tissue, may be able to restrict the development of competing fungi through prior and continuing utilization of free nutrients.

DISCUSSION

The experiments described in this paper have shown that mycelium of *C. herpotrichoides* remains alive in infested straw for periods of up to 3 years and can produce spores when placed under suitable conditions. This longevity could account for the survival of *C. herpotrichoides* in fields that had not carried a susceptible crop for a number of years and would explain, in part, why longer breaks in rotation are required to control eyespot than to control take-all. The protracted survival of *C. herpotrichoides* combined with an effective spore dispersal mechanism (Gregory & Stedman, 1958), and the production of secondary spores, greatly increase the effectiveness of a single piece of infested debris in distributing the fungus in both time and space. In contrast the ascospores of *O. graminis* do not appear to play any part in spreading the take-all disease (Garrett, 1956), this fungus being dependent upon mycelial growth for distribution.

Garrett (1950) suggested an ecological classification of root-disease fungi and distinguished between the unspecialized *soil-inhabiting fungi* and the more specialized *root-inhabiting fungi* characterized by 'an expanding parasitic phase on the living host plant and by a declining saprophytic phase after its death'. Although *C. herpotrichoides* does not attack the roots, but only the lower leaf-sheaths and the stems of plants, the saprophytic behaviour of this fungus is similar to the specialized *root-inhabiting fungi*, indicating that the criteria proposed for the ecological classification of the root disease fungi are also applicable to other groups of facultative parasites.

Saprophytic survival of *C. herpotrichoides* is undoubtedly an important part of the epidemic cycle of the eyespot disease. Further investigations are required to determine whether parasitic survival on weed grasses plays any significant part in the epidemiology of the disease.

The writer wishes to express his gratitude to Dr S. D. Garrett for his continued interest and helpful criticism; to Dr P. W. Brian for arranging for the antibiotic activity of *Cercospora herpotrichoides* to be tested; and to the Agricultural Research Council for the award of a Studentship during the tenure of which the greater part of this work was carried out.

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Studies on silvering disease of red beet

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(Received 8 April 1960)

SUMMARY

Observations and experiments were made on silvering disease of red beet during the period 1952-59. The disease is most prevalent on seed crops of the variety Cheltenham Green Top, but also affects other cultivars of *Beta vulgaris*, often only mildly. The causal organism is *Corynebacterium betae* sp. nov. which can be found in the tap-roots, stems and leaves of systemically affected plants causing veinal or uniform silvering of the leaves followed by wilting and death of the plant. External leaf infection gives rise to silvered lesions on the laminae or leaf margins.

About 1% of the seeds from infected plants contain the pathogen and these seeds give rise to plants showing systemic symptoms. The bacteria spread from such plants in the seedling bed during the autumn and winter, probably entering other plants through their leaf edges. Such spread can occur to distances of at least 50 yards, and the planted out seed crop may be up to 75% infected. The disease is readily transmitted on knives which have cut infected roots.

Seed treatment with streptomycin or erythromycin (200-400 µg/ml.) gives almost complete control of the disease. Mercurial dusts are less effective but have been found of some value in practice.

INTRODUCTION

Silvering disease of red beet was first recorded in Britain in 1945 (Moore, 1948) on a seed crop of the variety Cheltenham Green Top. Accounts by growers indicate that it may have been present before 1945, although not to a very great extent. Its origin is unknown and it has apparently not been seen in other countries.* Research on the disease was started by the writers in 1952 following preliminary studies by others (unpublished). By this time the disease had caused severe losses among seed crops of Cheltenham Green Top beet and many seed growers had ceased to grow the variety for this reason. Interim accounts of the research have been given in the Annual Reports of the National Vegetable Research Station (1953-60), and a description of the causal bacterium has also been published (Keyworth, Howell & Dowson, 1956).

Seed crops of the variety are mainly raised in Essex and Kent. In early August, stock seed is sown thickly in rows 9-12 in. apart and the young plants are known as

* Now reported to have been found in Eire (in 1958) on beet and mangel seed crops (McKay R., Loughnane, J. B. & McKay, J. (1960). Plant diseases in Ireland in 1958 and 1959. *J. Dep. Agric. Eire*, 56, 74).

'stecklings'. They normally remain in the 'steckling-bed' until February–March of the next year when they are dug up or ploughed out. Cheltenham Green Top is a long-rooted variety and the roots of the stecklings are trimmed before planting to about 5 in. long, by hand or by running a cutter under the soil along the rows. Plants with roots about the thickness of a pencil are regarded as most suitable for planting out, which is done mechanically or by hand at a spacing of 12–15 in. in rows 18–24 in. apart. The plants bolt in April–May and when they are about 12 in. high their tops are cut off to ensure a bushy growth. The 'ware' seed is harvested in September–October and after cleaning and grading is sold to growers of the root crop.

SYMPTOMS OF THE DISEASE

(a) *Systemic*

The first symptom appears in seedlings 6–8 weeks after sowing and is usually a silvering along the veins and adjacent laminal tissue of one or more leaves. Later, the silvering spreads to the whole lamina which may become thickened and brittle. Cracks appear in patches on the upper surfaces of the leaves, giving them a roughened appearance and exposing the underlying tissue (Pl. 1, fig. 2). The silvered leaves eventually wilt and die and finally the whole plant collapses.

Similar symptoms may appear after the plant has bolted, occurring either on one or more of the lateral branches (Pl. 1, fig. 1) or appearing relatively suddenly over the whole plant. The flower bracts and seed clusters may also be silvered. Wilting of the plant may ensue within a few days, but sometimes diseased plants remain alive for several weeks after the first appearance of silvering. No internal necrosis of the roots,* stems or petioles has been found to be consistently associated with the disease but the roots of the wilted plants may become rotted because of invasion by secondary organisms.

(b) *Localized*

Localized silvered areas are sometimes found on upper surfaces of the leaves of otherwise apparently healthy plants, especially when these are growing near to systemically affected ones. These silvered areas usually consist of roundish silvered spots 1–5 mm. diam. with a roughened cracked centre (Pl. 1, fig. 3), or of a silvered band 1–3 mm. wide round the edge of the leaf (Pl. 1, fig. 4). Little extension of the silvered spots has been noted, but the silvered areas around the leaf edges may extend inwards along the tissue beside the veins until the whole leaf becomes silvered (Pl. 1, fig. 5). By this time, other leaves on the plant may show systemic symptoms.

Anatomy of silvered leaves. The silvering symptom associated with this disease is unusual and the only close parallel is that of silver-leaf of plum, in which it was shown by Tetley (1932) that the silvering symptom was caused by the presence of air spaces between the cells of the leaf and especially beneath the epidermis. Evidence for the production of such air spaces was obtainable only by studying the development

* The term 'root' in this paper refers to the tap-root of the plant and not to the fibrous roots which grow from it.

of infected leaves. No similar studies have been made on the developing leaves of beet plants affected with silvering disease, but a comparison of sections of mature silvered leaves with those of normal leaves showed that the palisade cells in the former were rather widely separated and probably had air spaces between them. The chloroplasts were also light green in colour and appeared to be degenerating.

More detailed studies were made of the epidermal tissue on silvered lesions induced artificially by leaf infection. Surface views of the epidermis on and near one such lesion (Pl. 2, figs. 7, 8) showed that whereas the cells of the normal epidermis were polygonal and had no gaps between them, the epidermal cells on the lesions were rounded and separated by spaces which increased in size toward the centre of the lesion. The cuticle was also torn and the whole of the epidermis was ruptured at the centre of the lesion. The gaps between the epidermal cells and the rupturing of the cuticle will permit the entry of air, which presumably causes the silvering. Gaps between the epidermal cells, although less frequent, also occurred on leaves showing severe systemic symptoms.

Brooks & Brenchley (1931) showed that the silver-leaf symptoms on plum were caused by the translocation of a toxin from the fungus *Stereum purpureum* which was often present only at points considerably below the affected leaves. Several experiments were therefore made by the writers to induce silvering symptoms by injecting into beet plants filtrates from cultures in which the causal bacterium (see below) had been growing, but none of these attempts was successful.

The ultimate wilting and death of silvered plants may also be caused by a toxin. It is known (Pl. 2, fig. 6), however, that some of the vessels of silvered plants are plugged with bacteria. It is also possible that the gaps between the epidermal cells, described above, may contribute to the wilting effect. Normal seeding beet plants wilt very readily in hot weather and the presence of gaps in the epidermis, combined with extra air within the tissues, may increase the transpiration rate to the point where permanent wilting occurs.

THE CAUSAL ORGANISM

The disease was first thought to be caused by a virus, and this supposition was strengthened by numerous tests in which it was found to be transmissible by grafting portions of root together or by applications of infected sap to leaves and roots. In 1954, however, when it was shown that bacteria-free root sap would not transmit the disease and that silvering of leaf margins occurred, isolations were attempted from the silvered leaf edges. These yielded almost pure cultures of a species of *Corynebacterium* which was later isolated readily from other parts of affected plants. Inoculation experiments and re-isolations showed that this bacterium was the cause of the disease and it was described and named *C. betae* sp. nov. (Keyworth *et al.*, 1956).

Like other *Corynebacteria* the organism is a short Gram-positive rod ($0.8-1.6 \times 0.3-0.5 \mu$). It closely resembles *C. michiganense* (Smith) Jensen in many of its cultural characters, but differs from it in forming pale yellow transparent colonies on Yeastrel-glucose and meat-infusion-glucose-yeast agars. It also failed to infect tomato stems when inoculated into them through needle punctures.

LOCATION OF THE PATHOGEN IN THE PLANT

In sections of the roots and lower parts of the stem of heavily silvered plants, bacteria could be seen in a few of the vessels (Pl. 2, fig. 6). In other parts of the plants, however, they were not present in sufficient quantity for identification. Studies of the distribution of the pathogen were therefore made by culturing the organism from various parts of the plant. Portions of roots and stems were removed aseptically, usually after dipping the piece of tissue in ethanol and burning this off. When isolating from leaves and seed clusters, however, the tissue was merely washed in tap water so as to avoid killing subsurface bacteria. Consequently, some of the bacteria cultured may have been from the surface although other evidence suggests that they were, in the main, within the tissues.

(a) Tap-roots

Isolations from root tissues, both of seedlings and of maturing plants, yielded the bacteria with the greatest regularity and in the greatest numbers. It was usually possible to isolate *C. betae* from the roots even if the leaf symptoms were at a very early stage of development. The organism was apparently present in all parts of the tap-roots of severely affected plants.

(b) Aerial parts of bolting plants

C. betae was readily isolated from the stems of silvered plants, being present in July at distances of up to 65 cm. from the stem base. It was also present in the heavily silvered leaves. The seed clusters on such plants and their subtending bracts, although silvered, rarely yielded the pathogen. Attempts to isolate the organism from dry mature seed clusters were unsuccessful because of the presence of contaminants.

(c) Aerial parts of non-bolting plants

In tests of systemically infected plants *C. betae* was isolated readily from heavily silvered leaves if portions of their laminae were macerated in sterile water. The pathogen could also be obtained, although less readily, by placing drops of water on cracks in the leaf surface. Bacteria were also present in the petioles of heavily infected leaves and were isolated (though often sparsely) from young leaves showing only veinal silvering.

The systemically affected plants used in the above tests were usually found scattered within plots grown from infected seed (see below, p. 177). Near to them were often other plants on which the edges of the leaves were silvered and, as already noted, such edges yielded the pathogen as did also the silvered spots on the leaves of other plants. In addition, however, the bacterium was isolated from the edges, and sometimes from the laminae, of other nearby plants which were showing no symptoms of the disease.

Although in most of the tests the bacteria were isolated without surface sterilization of the leaves, some experiments were made in which the leaves were first washed in various disinfectants. These included 1 min. washings in mercuric chloride (1 %), Chlorox (11 % chlorine), streptomycin (400 µg./ml.) and Vantoc N* (10 %) and 1 hr.

* A quaternary ammonium commercial bactericide.

washing in Vantoc N (2 %). The pathogen was obtained from macerates of silvered edges after each of these treatments, and it was thus presumed to be present within the tissues, but conclusive proof of this was not obtained, as it was also possible to isolate the pathogen by dipping the treated edges in water and stroking them with a glass rod. This method was used because it was considered that simple washing might not remove all the bacteria from the surface. It is probable that the glass rod abraded the leaf edge slightly and allowed internal bacteria to escape, but the possibility cannot be ignored that the treatments did not in fact kill all the bacteria on the surface.

The apparent extreme susceptibility of the leaf edges to invasion by the pathogen remains unexplained. Since this invasion results in a more or less uniformly narrow band of silvering around most of the leaf edge it would appear to differ from that known to arise from invasion through hydathodes. In such cases (e.g. *Pseudomonas campestris* on brassicas and *Xanthomonas begoniae* on begonias) the leaf-edge invasion results in discrete lesions which spread inwards. The beetroot leaf has no obvious hydathodes.

SOURCES OF INFECTION

(a) Seed*

For some years, growers of seed crops have suspected that infected seed was the main source of the disease, because crops grown from seed obtained from different sources often showed great differences in disease incidence. Experiments were therefore made in 1952-54 to determine whether the disease was seed-transmitted.

In a preliminary trial in 1952-53, nine out of ninety-seven plants raised from seed taken from an infected crop became silvered while no diseased plants occurred among 100 plants grown from seed from a healthy crop.

In July 1953, a larger experiment was made, in which three plots (D 1-D 3) each 4×12 ft. were sown with seed from a 1952 crop that had contained 75 % infected plants, and three similar plots (H 1-H 3) were sown with seed taken from a 1952 crop showing almost no silvering. These six plots were grown within a small area, and were only $4\frac{1}{2}$ yards apart (the intervening gaps being sown with H seed). Another plot (C) was sown with H seed $\frac{3}{4}$ mile away to provide control plants raised in greater isolation.

The seedlings were lifted at the end of October and bedded in frames over the winter. Silvered plants were removed when seen. On 15 March the remaining plants were planted in the field in non-randomized plots where they were recorded for silvering until July. A summary of all the records is given in Table 1.

Subsequent experience has shown that, at the close spacings of the plots in this experiment, considerable inter-plot spread was likely to occur: the 32 % of silvered plants in plot H 2 is probably caused by such spread. However, the fact that the over-all figure for silvering on the D plots is clearly higher than that for the H and C plots strongly suggests that the disease was seed-borne.

A detailed account of attempts to control disease transmission in seed by various seed treatments is given below (p. 188). Some of these experiments can be regarded as

* The so-called 'seed' of a beetroot is actually a multilocular fruit (sometimes referred to as a 'cluster'). The term 'seed' in this paper refers to such fruits.

a direct extension of the 1953-54 experiment since they included comparisons of the infection occurring in plants grown from untreated seed obtained from diseased and healthy crops. A summary of the results of four such experiments is given in Table 2.

Table 1. *Seed transmission experiment, 1953-54*

Source of seed	Plot	No. plants	Cumulative percentage silvered						
			Oct.	Feb.	Mar.	Apr.	May	June	July
Diseased crop	D1	346	0.6	34	60	*	.	.	.
	D2	345	0	3	6	7	27	33	35
	D3	344	2.3	6	15	21	47	56	60
Healthy crop	H1	340	0	0	0	0	1	2	2
	H2	342	0	2	4	5	21	28	32
	H3	296	0	0	0.7	1	3	4	4
	C	619	0	0.2	0.3	0.5	1.5	2	2

* So many plants were affected in this frame that none was planted in the field.

Table 2. *Seed transmission experiments, 1955-56*

Expt.	Sowing date	Recording date	Plants from 'diseased' seed		Plants from 'healthy' seed	
			Approx. no.	% silvered	Approx. no.	% silvered
1	27 June 1955	20 Aug. 1955	4,300	0.93	4,600	0
2	11 Aug. 1955	20 Oct. 1955	3,300	2.03	3,900	0.05
3	12 Aug. 1955	24 Oct. 1955	2,000	1.00	2,000	0
4	17 May 1956	13 July 1956	4,900	0.43	4,500	0
Totals			14,500	1.01	15,000	0.01

The healthy seed in all four experiments was obtained from the same seed crop and was gathered from the centre of a field in which no disease had been seen. The diseased seed for Expts. 2-4 was all from the same, heavily infected, crop, and that for Expt. 1 was from another heavily infected crop.

The results show that, 8-9 weeks after sowing, 0.4-2.0% of the plants from diseased seed had become silvered whereas, except in one experiment, none of the plants from healthy seed had shown any infection. These experiments, therefore, also gave evidence that silvering was seed-borne.

Although less than 2% of the seed from infected plants appeared to be carrying the disease, many more infected plants eventually appeared during the growth of the crop, so the possibility was considered that a much larger proportion of seeds was infected but that the infection was not manifested until the plants matured. Subsequent experiments showed that this was not so, the increase in disease being due to spread from the original small proportion of plants growing from infected seed.

(b) Other sources

Alternative hosts. As there was no field evidence of the introduction of the disease from plants other than those in the genus *Beta*, no intensive search was made for

alternative host plants. When the disease was thought to be caused by a virus, sap from the roots of infected plants was rubbed on to the leaves of a range of virus indicator plants including spinach, *Chenopodium amaranticolor* and *C. album*. None of the plants became infected.

A more detailed study was made of the reaction of various cultivars of *Beta vulgaris* to the disease.

In 1953 systemic silvering symptoms were seen on a few seedling plants of mangel and Detroit red beet grown at the Research Station. In 1954 and 1955 leaves of sugar beet, mangel, spinach beet and Detroit red beet were successfully inoculated by applying to them either root sap from infected Cheltenham beet or cultures of *C. betae*. The inoculated leaves developed typical silvered lesions. Systemic symptoms were induced on sugar-beet seedlings by standing them in infected root sap.

In June 1954, thirty-seven varieties of red beet were inoculated by injecting seedlings with infected root sap and the plants were grown on in the field for observation. From one to seven plants of twenty-eight of the varieties developed symptoms during the following 8 weeks, but owing to the variability of the results the absence of infection in a variety could not be regarded as proof of resistance. Susceptible plants were found in all of the eight major red-beet types (Holland, 1957) that were tested, viz. Flat Egyptian, Egyptian globe, Detroit globe, Intermediate, Dwarf black long, Non-bleeding, Covent Garden and Cheltenham Green Top.

No surveys of commercial mangel seed crops have been made to determine whether silvering disease occurs frequently in them, but in July 1956 the disease was seen in two such crops in Essex, in one of which 7% of the plants showed symptoms. In the other (from different seed) a few scattered silvered plants were found. Dr R. Hull (private communication) states that the disease is very rarely seen in sugar-beet seed crops, a fact which may be related to the universal practice in Britain of dressing sugar beet seed with mercurial dusts.

While it is thus apparent that many varieties of beet are susceptible to infection by *C. betae*, no reports have ever been received of serious outbreaks of silvering in any variety other than Cheltenham Green Top. The present comparative freedom of other varieties from infection may be due to an inherent resistance to silvering, but it must be noted that the seed crops of the different varieties are normally grown at some distance from each other and they may thus have escaped the disease and the consequent build-up of infection in the seed stocks. The observations and experiments on mangel have shown, however, that this variety does possess an appreciable tolerance to infection. The silvering symptoms are usually mild and often unaccompanied by wilting. Thus, although silvering is rarely reported in crops of mangels, it is possible that the pathogen may be widely distributed within them. There is at present no field evidence that the amount of disease introduced into plantings of Cheltenham Green Top beet from nearby mangel crops is of any significance in comparison with that which arises from infected seed.

Plant debris. The pathogen may persist in diseased plant debris, especially if this is kept dry. Tests of dried silvered leaves demonstrated that they contained viable *C. betae* after 3½ years' storage in the laboratory. Seed dust from threshing operations

may be a potential contaminant of clean seed in the warehouse although this method of spread has not been established.

Soil. A few tests have been made on the persistence of infection in soil. In view of the long-term rotation of steckling beds and field plantings practised by growers, soil infection seems unlikely to be important unless it is very persistent and the evidence available suggests that it is not. Thus, following the lifting of the plants from the frames in the 1953-54 seed-transmission experiment, eighty healthy seedlings were planted in each of three frames that had contained respectively 60, 20 and 7% of infected plants. None of the new plants showed any symptoms after a period of 3 months. In greenhouse experiments several attempts were made to infect young seedlings by watering the soil with suspensions of *C. betae* or with infected root-sap, but no disease occurred.

Experience on commercial holdings suggests that, compared with the introduction of the disease in seed, no other source of infection is of great significance at present. If seed-borne infection is reduced or eliminated, however, other sources of the disease may become of more importance.

EXPERIMENTAL TRANSMISSION OF INFECTION

(a) *Infection of tap-roots*

Plants were readily infected by inoculating their tap-roots. In one such experiment thirty-two partly mature healthy Cheltenham Green Top beet, bearing tap-roots 4-5 cm. diameter, were used and a hole $\frac{1}{2}$ cm. diam. and 2 cm. deep was bored in each root. Into each of sixteen roots was placed $\frac{1}{2}$ ml. of a heavy suspension of *C. betae* in nutrient broth and the remainder received the same quantity of sterile broth, the holes in all the roots being plugged with a core of healthy root tissue. The plants were then grown in pots of soil in the glasshouse. Twenty-one days later, eleven of the sixteen inoculated plants showed veinal silvering on their young leaves, indicating systemic infection by the pathogen, and after 31 days all the inoculated plants were showing symptoms. None of the controls became infected.

This method of inoculation was subsequently used in many experiments (e.g. to test the pathogenicity of isolates from various sources) and consistently gave typical systemic infection. In other experiments young seedlings were infected, usually by injecting a small quantity of bacterial suspension into the hypocotyl. Most of the plants inoculated in this way developed systemic symptoms and the method was used for producing infected seedlings for field experiments.

Infection was also transmitted to tap-roots when they were inoculated with sap from infected roots or with inserted portions of infected root. The pathogen was also transmitted very readily on knives that had been used to cut infected roots. Thus in one such test 10/10 plants developed systemic symptoms within 6 weeks after their tap-roots (4-5 cm. diam.) had been notched with a scalpel that had been infected on each occasion by cutting a similar notch with it in an infected root. None of the ten control plants showed symptoms. In another test 29/30 inoculated plants contracted the disease compared with 2/50 controls.

The cutting of such notches in tap-roots is a routine commercial measure for the inspection of roots for colour, etc., when selecting mother plants for stock-seed raising. The practice may obviously increase the amount of infection in the primary stock very considerably.

Attempts that were made to find a suitable dip treatment for sterilizing knives were not successful, infection still occurring after contaminated knives had been dipped for periods of up to 1 min. in formalin (10%), ethanol (95%), acetic acid (50%), streptomycin sulphate (300 µg./ml.), ethyl mercuric phosphate (200 µg./ml.) or Vantoc N (400 µg./ml.). The knives could be sterilized by heating them or by burning off the ethanol in which they had been dipped, but neither method was considered suitable for use in the field.

The disease was also transmitted from root to root by allowing a rabbit to bite first a diseased root and then a healthy one. In one experiment ten out of fourteen roots inoculated in this way became infected, compared with 0/14 control roots. The roots of seed-plants are frequently bitten by hares in the field and this may therefore spread the disease.

No transmission of infection occurred when species of slugs, millipedes and pygmy mangel beetles were fed on diseased roots and then on healthy roots. Tests were made to determine whether the disease was transmitted from root to root by the handling that took place in the course of an experiment or that occurred when stecklings were lifted for planting in the field. These showed that very little, if any, transmission occurred when the roots were handled in the normal fashion but 5/25 treated plants became infected when the roots were rubbed in such a way that the surface was abraded. This mode of transmission thus seems unlikely to be of importance in practice.

(b) *Infection of leaves*

In the earlier experiments the upper surfaces of young leaves were rubbed with a finger that had previously been dipped in a suspension of *C. betae* or rubbed across a culture of the bacterium. Silvered lesions appeared on the leaves 5–10 days after inoculation. The lesions were usually circular (diam. 0.5–3.0 mm.). They were more numerous and often confluent if the leaf surface had been abraded with celite immediately before the inoculation (Pl. 2, fig. 9). When the cotyledons on very young seedlings (i.e. without any primary leaves) were inoculated in the above fashion they became heavily silvered.

In later tests both surfaces of leaves were sprayed with bacterial suspensions, with or without leaf abrasion, and the plants were then enclosed in hurricane-lamp-glass moist chambers for 2–3 days. Numerous lesions appeared on both the upper and lower surfaces of the abraded leaves but inoculations without abrasion gave very erratic results, there being sometimes 1–10 lesions per leaf but occasionally none at all. In an effort to reproduce the types of damage that might occur in the field, holes of various sizes were pricked or punched in leaves before they were sprayed. The leaf tissue around the edges of such holes frequently became silvered and occasionally the infection spread along the veins near the holes. The symptoms resembled those found occasionally in the field around holes in leaves made by insects or birds.

It was also thought possible that the leaves on seedlings in the field might be subjected to some abrasion from sand particles blown against them and several experiments were made to determine whether such abrasion would encourage the entry of bacteria. The following is a typical example.

Sand abrasion experiment. To test the possible field effect of wind-blown sand particles in inducing abrasions predisposing to infection, sand was blown against the leaves of young beet plants, using a dusting apparatus giving three air velocities (approximately 7.5, 15 and 30 ft./sec.) and applying two sizes of particle (30-40 mesh and < 80 mesh per inch). Potted plants bearing seven to ten leaves were held on their sides in the horizontal air-stream for periods of 12 sec. so that the sand particles impinged approximately at right angles to their leaf surfaces. The plants were then sprayed with *C. betae* and kept under hurricane glasses for 24 hr. Details of the treatments and the results obtained are given in Table 3.

Table 3. *Sand abrasion experiment*

	Wind speed (ft./sec.)	Size of sand (mesh/in.)	Bact. spray	No. plants	Av. no. lesions per plant
Uninoculated controls	0	.	—	6	0
	30	30-40	—	3	0
	30	< 80	—	3	0
Inoculated with- out abrasion	0	.	+	12	4
	30	.	+	6	0
Inoculated and abraded	7	30-40	+	6	2
	15	30-40	+	6	4
	30	30-40	+	6	40
	7	< 80	+	6	12
	15	< 80	+	6	42
	30	< 80	+	6	206

It is apparent that certain of the treatments greatly increased the numbers of lesions produced by the subsequent inoculation and that the effect was directly related to the wind speed and to the number of particles impinging on the leaf. The sand treatment alone had no macroscopic effect on the leaves, nor could any injury be detected beneath the microscope but it is presumed that the sand ruptured the cuticle and facilitated the entry of bacteria.

Leaf-edge infection

Since the earlier work on leaf infection, field studies have shown that the invasion of leaf edges is an important mode of entry of the pathogen. Only one attempt has, as yet, been made to induce such infection artificially and this was unsuccessful, probably because, although the plants were kept enclosed for 2 days, their leaves were not sufficiently wet for edge infection to take place.

Tests of leaf-to-leaf transmission by insects and other animals

As already noted, silvering was occasionally seen around holes in the otherwise healthy leaves of young plants in the field. Such plants were usually not near to any other silvered plants, suggesting that the infection had been transmitted by the insect or other animal that had made the holes, presumably at a much earlier stage in the plant's growth. A series of experiments was made, therefore, in which insects were fed on silvered leaves and then transferred to young healthy leaves, but no transmission was obtained using *Myzus persicae*, *Aphis fabae*, species of pygmy mangel beetles, earwigs, flea beetles, slugs and millipedes, and several unidentified caterpillars.

Systemic infection following leaf inoculation

In some of the experiments in which leaves or cotyledons were inoculated the plants were retained for varying periods to determine whether the pathogen invaded the plants systemically. Typical systemic symptoms were seen on several occasions on the new uninoculated leaves of such plants, but the results were very variable. In one experiment, two leaves on each of thirty plants were inoculated with bacterial suspensions. After 14 days twenty-four plants bore silvered spots on their leaves and four of these plants had become systemically infected 7 weeks later. In another experiment in which the cotyledons of twenty very young seedlings were inoculated, systemic symptoms appeared on the newly developing leaves of ten of the plants after 4 weeks. None of the control plants showed infection in either experiment.

Later experiments failed to give such a high number of systemic infections. Thus in one case only 3/100 inoculated plants showed systemic symptoms after 3 months. Further experiments are obviously necessary to determine the conditions under which systemic invasion will most readily occur.

Attempts to infect seed

Partly to test the possible natural mode of infection of beet seed and partly in an attempt to obtain large numbers of systemically infected seedlings, a number of tests were made of the effect of soaking seed in suspensions of *C. betae*. In one experiment, seed was soaked overnight in a concentrated bacterial suspension and in $\times 50$ and $\times 2500$ dilutions of this suspension. Two out of fifty-five plants raised from seed soaked in the heaviest suspension showed silvering 6 weeks after sowing. None of the other plants became infected. Two other tests were made in which dry seed and seed soaked in water for the previous 24 hr. were immersed in bacterial suspensions for 24 hr. In an attempt to force bacteria into the seed-coat, half of the seed in each batch was soaked under reduced air pressure. In no case did any of the plants grown from the seed show symptoms of silvering.

These results are similar to those of Grogan & Kendrick (1953), working with *C. michiganense* on tomato, who were unable to obtain more than 1% infection of tomato seedlings by dipping seed in a suspension of the pathogen.

SPREAD OF THE DISEASE IN THE FIELD

*A. Observations on commercial holdings**(1) In steckling beds*

As already described, the main source of infection of the new crop is the relatively small number of systemically infected seedlings, arising from infected seeds, which can be found scattered through the steckling beds some 6–8 weeks after the beds are sown. The numbers of such plants may increase slightly during the autumn and other plants may be found which bear silvered lesions on the margins or elsewhere on the surface of their leaves.

Symptoms cannot be detected in winter, when the leaves of the stecklings become dark red and new leaf production almost ceases. In plants retained in the steckling bed, new growth starts in March or early April, by which time many of the plants which showed systemic symptoms in the autumn are dead, but such symptoms appear on the young leaves of nearby plants and become progressively more obvious until by the end of April very many of the plants may be seen to be infected. When, as in commercial practice, the plants are transplanted to the field in February–March this checks growth and causes a further suppression of symptoms which may not appear until May when the plants are starting to ‘bolt’. This is usually the first occasion on which the grower sees the disease.

(2) In maturing seed plants

It seems likely that the major spread of the disease in the seed crop occurs when the plants are close together in the steckling bed. Some further spread probably occurs, however, when the roots are trimmed for planting since, as already described, the disease can be spread on infected knives. Commercial plantings frequently show long ‘runs’ of adjacent silvered plants in individual rows, with few or none in the rows on either side. This is thought to be due to the planting of such ‘runs’ with stecklings from an infected group in the plant-bed, combined perhaps with the spreading of infection when the roots are trimmed. When the plants are 12–15 in. high their tops are cut off to encourage the growth of side shoots but experiments have failed to show that this operation transmits the disease. It has been demonstrated, however, by planting infectors in a healthy seeding crop, that spread of infection can occur during the summer from one seeding plant to another.

Most of the plants showing symptoms in the spring die as the season progresses, but other plants develop symptoms later and some of these survive and produce seed.

(3) In the root crop

The seed for the root crop is sown in June and the roots are harvested in the autumn. Silvering disease is rare in the root crop; a survey of some 60 acres of root crops of Cheltenham Green Top beet made by the authors in September 1953 showed that an average of one in 10,000 of the plants was silvered. Experimental sowings in the summer have shown that up to 1 % of the young seedlings may show symptoms within 8 weeks after sowing but it is presumed that, in commercial fields, most of these die

and no visible spread occurs, resulting in the very small amount of disease seen in September.

To determine whether any spread of the disease could take place in a root crop, thirteen silvered plants and thirteen healthy plants (each at some distance from a silvered plant) were marked in October 1953 with canes in a commercial root crop. Ten adjacent, apparently healthy, plants were lifted from around each marked plant and the two lots of 130 plants were bedded in separate covered frames at the Research Station, each group of ten plants occupying a short row. Between each pair of rows were planted ten plants known to be healthy, to detect any spread that might occur in the frames. Some plants became infected with downy mildew during the winter and were discarded and six of the plants showed silvering in March. The remaining plants were grown on in the field and recorded at intervals until the end of June. The numbers of silvered plants in each group are shown in Table 4.

Table 4. *Spread of disease in a root crop*

Group no.	Taken from around silvered plants		Taken from around healthy plants	
	No. plants recorded	No. silvered	No. plants recorded	No. silvered
1	4	0	2	0
2	9	3	6	0
3	3	1	4	0
4	6	3	6	0
5	6	2	10	0
6	8	5	4	0
7	3	0	8	2
8	5	4	7	0
9	1	0	1	0
10	4	1	7	0
11	5	3	6	0
12	4	1	9	0
13	5	2	5	1
Totals	63	25	75	3
Controls	84	1	99	0

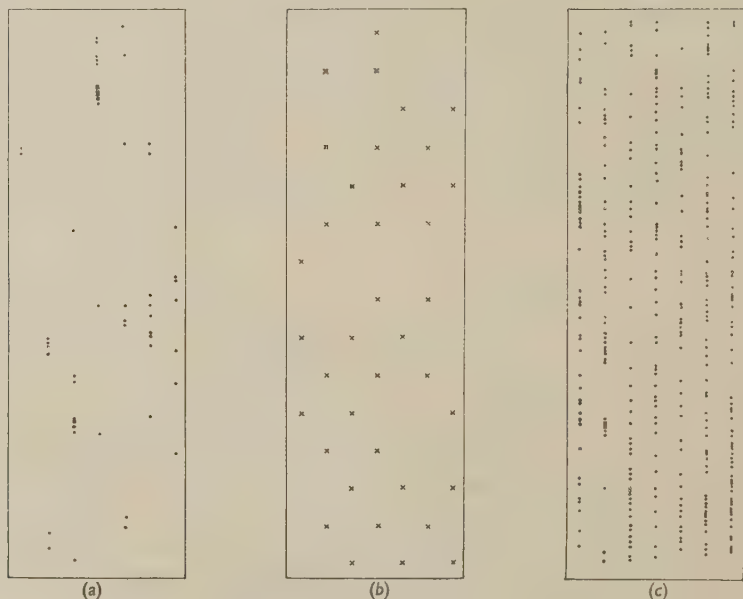
The results show that ten of the thirteen groups of plants lifted from around silvered plants in the root crop contained a total of twenty-five silvered plants, whereas only two of the groups lifted from around healthy plants contained any silvered plants (total 3). The very low disease incidence in the control plants, included to detect spread in the frames, showed that such spread was negligible. It may thus be concluded that spread of the disease had occurred in the root crop from silvered plants to healthy plants, although these appeared healthy when collected.

B. *Experimental study of spread in steckling beds*

During the autumn of 1958, observations were made on the spread of the disease within steckling beds sown with seed from infected crops, and also from such beds to other healthy ones. The wet weather in August–November appeared to favour the spread of silvering.

(1) *Spread within a steckling bed*

At Wellesbourne, two plots, each 22 ft. long and containing seven rows 1 ft. apart, were sown on 15 August. Plot A was sown with seed obtained from silvered plants and plot B was sown with commercial seed containing less infection. Some plants in each plot showed systemic silvering from mid-September onwards. At the end of September, silvered edges were seen on the leaves of some otherwise normal plants. Isolations made from the silvered edges by macerating portions of them in sterile water (without surface sterilization) yielded *C. betae* but so also did four out of six apparently normal leaf edges. A more detailed study was therefore made of the distribution within the plots of leaves bearing bacteria.



Text-fig. 1. Spread of infection in a steckling plot (sown 15 August 1958). (a) Plants showing systemic infection on 15 October. (b) Positions of plants from which the pathogen was isolated, from leaf edges, on 15 October. (c) Plants showing systemic infection on 1 May 1959.

On 15 October fifty leaves were picked (without prior inspection) from each plot, at points marked by canes spaced 18 in. apart in each row. A strip approximately 3 mm. wide and 3 cm. long was cut from one edge of each leaf and macerated in sterile water. Isolations were made from the individual macerates using suitable precautions to avoid transference of bacteria from one to another.

Thirty-seven of the fifty leaves from plot A yielded the pathogen. Of these, only nine had silvered edges. The organism was isolated from sixteen of the fifty leaves

from plot B, two of them having silvered edges. The distribution of the systemically infected plants in plot A on 15 October and the positions of the leaves from which *C. betae* was isolated are shown in Text-fig. 1, together with a map of the distribution of the systemically infected plants on 1 May 1959.

It is obvious that, by 15 October, the pathogen had become very widely distributed throughout the plots and was not confined to plants near to those showing systemic symptoms. It should also be noted that these latter plants occurred in groups, suggesting that some spread, giving rise fairly quickly to systemic symptoms, had occurred before 15 October. It seemed likely, therefore, that during the 7 weeks in which the plants had been above ground, a relatively localized spread of infection had been succeeded by a much more widespread distribution of the pathogen, causing a large amount of systemic infection in the following spring.

To test the possibility that the presence of the pathogen in or on the edges of the apparently healthy leaves was not caused by spread from the systemically infected plants but by the accumulation of bacteria in the leaf edges of plants that were already internally infected, isolations were made from various parts of systemically infected plants and from others showing only leaf-edge silvering or no symptoms. The pathogen was present in the roots and most of the petioles and leaves of the plants showing systemic symptoms but in plants showing leaf-edge silvering or no symptoms the bacterium was obtained mainly from the leaf edges, occasionally from the laminae and petioles and never from the roots. It thus appeared that the latter plants had become infected from external sources. The bacteria were presumed to have been transmitted from the leaves of the systemically infected plants and could be isolated from drops of water left overnight on cracks in heavily silvered leaves. They were never obtained in very large numbers, however, and it seems likely that the method of isolation did not adequately reproduce the conditions under which the bacteria were liberated in the field.

(2) *Spread from one steckling bed to another*

An experiment was started on 18 August 1958 to investigate the spread of the disease from infected steckling beds to adjacent and distant healthy ones. There were six infector plots (S) sown with seed from silvered plants and situated 100 yards apart near the edge of a 7-acre field. Next to each S plot was an H plot sown with healthy seed (that had also been treated with erythromycin and Harvesan). Six other H plots were sown with this seed, each at a distance of 50 yards from any S plot.

By 11 November, 4–14% of the plants in the S plots were silvered but, with the exception of two plants, none of the plants in the H plots showed symptoms. The pathogen was, however, isolated from the edges of seven out of forty leaves picked from the H plot that was adjacent to the most heavily diseased S plot.

Further records of the numbers of silvered plants were made in the spring. By 21 April, over 50% of the plants in the S plots and 5–16% of those in the adjacent H plots were silvered. Infected plants were also present in four of the distant H plots in amounts varying from 0.1 to 10%. Two of the distant H plots, situated in the

north-west area of the field, contained no infected plants. The two most heavily infected plots were in the south-east area of the field.

These results show that the pathogen had spread during the autumn and winter to distances of at least 50 yards, although the silvering resulting from this spread did not become apparent in the H plots until the spring. Most spread occurred in a south-east direction and this may be related to the prevalence of north-west winds in the area during 1958.

CONTROL OF THE DISEASE

As the pathogen was found to be seed-transmitted, and there appeared to be no other important sources of infection, most of the work on the control of the disease was concentrated on attempts to disinfect the seed. As already noted, seed stocks harvested from silvered plants usually contained only 1% or less of infected seeds. Assessments of any reduction in this small percentage required the inspection of a large number of plants and the continuance of the trials for several years. These were of two main types, viz. (a) short-term trials which were maintained for up to 3 months after sowing and in which the silvered plants were presumed to have arisen directly from infected seeds, and (b) extended field trials in which the plants were retained over the winter and then grown to maturity next season to assess the amounts of disease in the final seed crops. In these latter trials the effects of the treatments were enhanced because of the spread of silvering within the steckling plots in which the disease had not been controlled. To avoid spread of disease from such plots to others, however, they had to be widely separated and the numbers of treatments and replications reduced in consequence.

(1) *Heat treatment*

Preliminary studies were made on the possibility of controlling seed infection by heat treatment. Tests, in which Difco nutrient-broth cultures of *C. betae* were enclosed in capillary tubes and heated for 10 min. in water at various temperatures, showed that the thermal death-point of the bacterium was 55° C. Seed of 80–90% initial germination showed little or no loss in viability when heated for 20 min. in water at 60° C. but temperatures above this caused a progressive reduction in germination, and some older seed was damaged at 60° C. Owing to the success of other methods of seed disinfection, the work on heat treatment was not continued.

(2) *Chemical disinfection*

Shortly before the studies on this subject were started, a number of workers elsewhere had reported that the seeds of various plants infected with bacteria could be disinfected by soaking them in streptomycin solutions (Ark, 1947, 1954; Klinkowski, Köhler & Shrödter, 1955). The use of antibiotics of this type appeared to have several advantages: the compounds were often highly lethal to bacterial plant pathogens; they were relatively non-toxic to plants; and they penetrated plant tissue.

In vitro tests showed that streptomycin sulphate, erythromycin, ethyl mercuric phosphate and a number of proprietary quaternary ammonium surface-active agents,

killed *C. betae* at concentrations below 10 $\mu\text{g.}/\text{ml.}$ These compounds and some others were then used for seed treatment.

The location of the pathogen within the 'seed' was unknown, but it was assumed that it would be necessary for the disinfectants to penetrate the seeds. Seeds were therefore steeped for at least 18 hr. in bags made of curtain netting of about $\frac{1}{8}$ in. mesh, and agitated frequently at the start of the process to ensure that most of the air within them was liberated. The seeds were then spread out and allowed to dry before being sown in field plots. As preliminary tests showed that water soaking had no effect on silvering, unsoaked silvered seed was used for the control plots in the later experiments. Some tests were made with organo-mercurial dusts which were mixed with the seed shortly before sowing at the rate of 5 g. per 1 lb. seed. The seed was sown by hand or with a Planet drill, precautions being taken to avoid transference of any dust to undusted seed. If 'healthy' untreated control seed was used it was sown first and the untreated 'silvered' seed was sown last.

Short-term trials

These trials were sown in June–August and records were made of any plants showing systemic silvering during the following 8–10 weeks. A summary of the results from 5 years' trials (1955–59) is given in Table 5.

Table 5. *Summary of results of short-term seed treatment tests (1955–59)*

Compound	Rate (active ingredient) ($\mu\text{g.}/\text{ml.}$)	No. of trials	Approx. total no. plants	No. silvered	% infection
Streptomycin sulphate	150	4	6,500	2	0.03
Streptomycin sulphate	300	5	17,800	0	0.0
Erythromycin	200	2	4,000	0	0.0
Ethyl mercuric phosphate	100	3	5,500	0	0.0
Fixanol	200	2	2,700	16	0.60
Vantoc N	200	2	3,300	5	0.15
Neomycin	200	1	1,300	11	0.85
8-hydroxy quinoline potassium sulphate	200	1	800	16	2.00
Malachite green	200	1	2,400	14	0.58
Harvesan	*	6	19,900	21	0.13
Agrosan	*	3	9,200	6	0.06
Leytosan	*	3	8,900	9	0.10
Nil (control)	—	11	33,800	324	0.96

* Used at the rate of 5 g. of proprietary material per 1 lb. seed.

It is clear, from these results, that streptomycin, erythromycin and ethyl mercuric phosphate gave the best control of infection. Ethyl mercuric phosphate caused some reduction in germination, however, and also has a very high human toxicity. For these reasons it was not included in further tests. Erythromycin and streptomycin, although available for experimental work, were not then obtainable by growers. Thus, although it was decided to make more extensive trials with both compounds, it was thought advisable to include in such trials a material which was available commercially,

and Harvesan was selected for this purpose. The other two mercurial dusts were apparently of the same order of effectiveness and Harvesan was used merely because it was the first one to be tested.

Extended field trials

There were two of these trials, and in both of them the steckling beds grown from treated and untreated seed were located as far apart as possible in an attempt to reduce the spread of the disease from one to another.

The first trial, made in Essex in 1955-56, consisted of a simple comparison between a crop from untreated seed and another one grown from the same seed after it had been treated with streptomycin sulphate ($300\text{ }\mu\text{g./ml.}$). The two steckling beds were $\frac{3}{4}$ mile apart, and in March 1956 the stecklings from each bed were used to plant 4 acres of seed crop. The two crops were in the same field, but about 50 yards apart.

On 11 JUNE, sample counts of silvered plants were made in the untreated crop and a complete count was made of half of the treated crop. The results are given in Table 6.

Table 6. *Silvering in commercial crops grown from treated and untreated seed*

Seed treatment	Disease record on 11 June		
	No. plants inspected	No. silvered	% disease
Streptomycin	17,580	24	0.14
Nil	1,801	1192	66

The untreated crop yielded $1\frac{1}{4}$ cwt. seed per acre and the treated crop 11 cwt. per acre. The treatment, which cost under £2, thus resulted in a cash return of over £600.

Table 7. *Effect of seed treatment on silvering in mature seed crops*

Site of steckling bed	Percentage silvered plants from seed treated with			No treatment
	Streptomycin	Erythromycin	Harvesan	
N.V.R.S., <i>a</i>	0	0	3.7	27.6
<i>b</i>	0.6	0	5.1	32.6
Paglesham, <i>a</i>	0	0	23.3	9.8
<i>b</i>	1.1	0	3.5	10.1
Luddington	0	0	7.0	26.1
Average	0.3	0	8.5	21.7

In a further trial, started in August 1956, seed obtained from silvered plants was treated with streptomycin ($400\text{ }\mu\text{g./ml.}$), erythromycin ($200\text{ }\mu\text{g./ml.}$) or Harvesan (5 g./lb.) and, together with untreated seed, was sown in three localities. At the Research Station and at the N.V.R.S. substation at Paglesham, Essex, two plots 20 yards apart were sown with each type of treated seed. Each pair of plots was located at least $\frac{1}{4}$ mile from any other pair. At the Luddington Experimental Horticulture Station near Stratford-on-Avon, four single plots $\frac{1}{4}$ mile apart were each sown with seed receiving one of the treatments. The stecklings were grown over the winter on these sites and,

in early April 1957, 200 plants were removed at random from each of the twenty plots and planted at the Research Station in plots about 20 yards apart. The percentages of silvered plants that had been recorded in each plot by 21 June are given in Table 7.

The amount of disease in the control plots was not as great as had been seen on other occasions in maturing seed crops but was sufficient to indicate the high degree of control obtained by the streptomycin and erythromycin seed treatments (especially the latter). The Harvesan treatment, although giving a considerable reduction in average disease incidence, was much less effective. At one site (Paglesham, *a*) the Harvesan-treated plot showed more silvering than the control, presumably owing to extra spread of infection within the original steckling plot.

Other farm trials

Both streptomycin and Harvesan were tested on several farms during 1957-59 but without control plots. In 1957 and 1958, crops grown from seed treated with streptomycin usually contained less than 1% infection and those grown from seed treated with Harvesan contained less than 5% infection. In 1959, however, following the wet autumn of 1958 when spread of the disease in steckling beds had been considerable, up to 25% infection was seen in crops grown from seed treated with Harvesan and up to 5% in crops grown from streptomycin-treated seed. Seed treatment with mercurial dusts did not appear, therefore, to be a consistently satisfactory method of control.

Since September 1958 growers have been permitted to use commercial streptomycin formulations for agricultural purposes. Much of the seed sown for the 1960 crop has been treated with one such formulation.

Further studies on the mechanism of seed disinfection

It was clear, from the short-term and field trials, that the dry mercurial seed dressings, although not so effective as streptomycin, gave some reduction in seed infection. It was considered that they might therefore have some ability to penetrate the seed. One of the dressings used had two active ingredients, one (tolyl mercury acetate)

Table 8. *Test of constituents of a mercurial dressing*

Test no.	Percentage infection after seed treatment with			
	Complete dressing	T.M.A.	E.M.C.	No treatment
1	0	0.04	0.04	0.43
2	0.25	0.15	0.23	0.77
3	0.07	0.16	0.09	1.04

being non-volatile and the other (ethyl mercury chloride) being volatile. To test whether the volatile component was the effective one, three short-term experiments were carried out in which seed was dressed (at 5 g. per lb.) with the mixture and with each of the components separately, in the proportions in which they occurred in the mixture. The results of these experiments are given in Table 8.

It appeared from these results that both constituents of the dressing caused some

reduction in disease and the effect of the complete dressing could not be ascribed solely to the volatile component.

In another experiment the effect of soaking seed in streptomycin (150 $\mu\text{g./ml.}$) for 10 min., 1 hr. and 24 hr. was tested. All eliminated infection (which occurred to the extent of 0.9% in the control plants). On the other hand, dusting the seeds with a powder containing 7% streptomycin did not eliminate the disease nor, as shown in Table 5, did an 18 hr. soak in 200 $\mu\text{g./ml.}$ solutions of Fixanol, Vantoc N or Malachite Green, all of which killed the pathogen in culture at concentrations of less than 5 $\mu\text{g./ml.}$ These latter results are considered to demonstrate that the pathogen was not borne superficially on the seed. Streptomycin was presumably active after the long-soak treatment because it penetrated the seed, although the effectiveness of the 10 min. steep in the material is less easy to explain.

Development of strains of Corynebacterium betae resistant to streptomycin

It is well known that bacteria treated with streptomycin or other antibiotics may develop strains which are resistant to the effects of the compound. In several of the farm trials a few silvered plants occurred in crops grown from seed treated with streptomycin. Cultures of *C. betae* were therefore obtained from such plants growing on farms and the bacteria were subjected to streptomycin toxicity tests in comparison with cultures from silvered plants not grown from treated seed. All the isolates were killed by the same concentration of streptomycin, and there was no evidence that any of them were resistant to its effects. It may thus be presumed that the silvered plants occurring in the crops grown from treated seed had arisen from seeds that had escaped the treatment or that they had become infected from outside sources. It is clear, however, that further tests should be made in succeeding years so that, if resistant strains of *C. betae* are found, an attempt can be made to kill them by simultaneous treatment with two effective compounds.

DISCUSSION

Silvering disease of beet has some affinities with certain other plant diseases caused by *Corynebacteria*, but differs from them in several respects. The ready transference of the pathogen on knives which have cut infected roots is also found in the transmission of *C. sepe-donicum* in potato, and the leaf necrosis and seed transmission phenomena are reminiscent of bacterial canker of tomato caused by *C. michiganense*. On the other hand, the silvering symptom is unique within this group of diseases as is also the apparently low concentration of the bacteria within the plant (especially in the aerial parts). Both phenomena suggest that the pathogen produces a highly virulent toxin but no proof of this was obtained.

Two noteworthy features of the disease are the low incidence of infection in seed from diseased plants and the considerable spread of the disease within the steckling beds.

The evidence in the present study on the percentage seed transmission of the disease has been examined very critically, especially in view of the varying reports on this

subject in the case of bacterial canker of tomato (e.g. Blood (1937), Ark (1944) and Grogan & Kendrick (1953)). Estimates of percentage transmission based on counts of infected plants are open to the objection that there may be considerable delay in symptom expression. The experiments on silvering disease have, however, given clear evidence that the gradual increase of infection in seed crops is caused by spread of the disease and not by a delay in the appearance of symptoms. The way in which this spread occurs is not clearly understood, especially in respect of the mode of entry of the pathogen into the plant. The edges of the leaves are apparently very readily invaded and they may be the main entry point for the bacteria. On the other hand, the very rapid development of systemic symptoms in the autumn of 1958 on some of the experimental plots suggests that there may be some other means of transmission, perhaps from root to root. These phenomena obviously require further study.

If the present effectiveness of seed treatment with streptomycin and other compounds is maintained, the commercial control of the disease seems to be assured. Continuing observations on this matter will be made, however.

Grateful thanks are extended to all those growers and seedsmen who have given facilities for field trials and observations on the disease; also to those officers of the N.A.A.S. who have collaborated in some of the experiments and the several commercial firms who have supplied compounds for test as seed disinfectants. Special mention must be made of the help and advice given by Dr R. A. Shooter of St Bartholomew's Hospital, London, in the studies on the sensitivity of the pathogen to antibiotics and of the encouragement and help during the whole of the work given by Dr W. J. Dowson.

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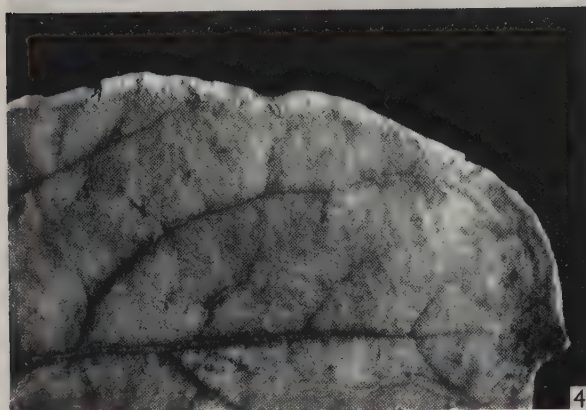
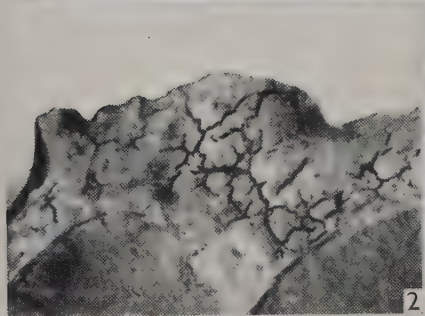
EXPLANATION OF PLATES

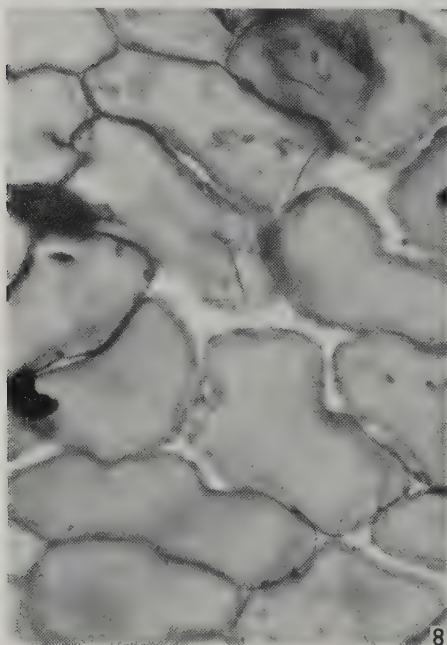
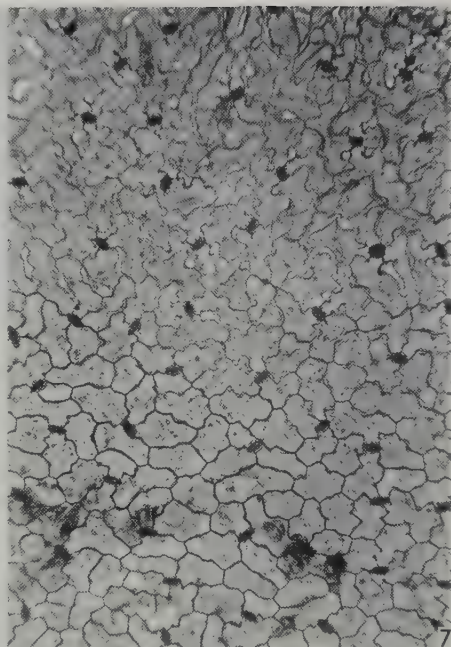
PLATE 1

- Fig. 1. Lateral shoot on seed-plant showing veinal silvering symptoms.
Fig. 2. Cracks on the surface of a heavily silvered leaf.
Fig. 3. Silvered spots on seedling leaf, arising from natural spread of bacteria in a field plot.
Fig. 4. Silvered edge on a seedling leaf, arising from natural spread of bacteria in a field plot.
Fig. 5. Extension of silvered area from the edge toward the centre of the lamina of a seedling leaf.

PLATE 2

- Fig. 6. *C. betae* within the vessels of the lower part of the stem of a silvered seed-plant.
Fig. 7. Surface view of the epidermis on a silvered spot showing transition from normal tissue to tissue with large intercellular spaces.
Fig. 8. Epidermis on silvered spot showing rupture of cuticle.
Fig. 9. Artificial infection of leaves by rubbing them with sap from infected roots: (a) healthy leaf; (b) rubbed with sap; (c) rubbed with sap, using celite.





**Observations on the biology of chrysanthemum eelworm
Aphelenchoides ritzema-bosi (Schwartz) Steiner
in florists' chrysanthemum**

I. Spread of eelworm infestation

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(Received 16 May 1960)

SUMMARY

The chrysanthemum eelworm moved in sand and in a clay soil at much the same rate as other eelworm species so far studied. Leaf washing and soil examination in infested chrysanthemum plots showed that the eelworm never spread laterally through the soil or from leaf to leaf more than to the second row of plants from the source of infestation, a maximum distance of about 30 in. At Rothamsted and in the National Chrysanthemum Society's variety trial the number of infested varieties increased greatly in September, during periods of heavy mists. Experiments with isolated leaves, artificially infested and kept under either dry or humid conditions suggested that eelworms were more active in humid conditions. They invaded isolated leaves within 15-30 min. In pot experiments, eelworms added to soil invaded chrysanthemum plants inducing symptoms in 18 days: 26 days after such inoculations no eelworms could be detected in the soil. Few eelworms persisted in fallow soil in pots after 32 days. No eelworms were found after the end of November in soil taken from an infested field plot at intervals between August 1959 to January 1960. Attempts to infest plants with eelworms from stored soil taken from highly infested plots failed. Examination of stools during the winter showed that many eelworms were present in the green tissues. It is concluded that infestation from eelworms which persist over the winter in soil is slight and that the main source of the pest is the chrysanthemum stool.

INTRODUCTION

A knowledge of the methods of spread of chrysanthemum eelworm is a necessary prerequisite to the study of control. Voss (1930) suggested that new infestations arose from dormant eelworms which overwintered in dead infested leaves. Stewart (1921) briefly mentioned the survival of adult eelworms in leaves in the soil. Wilson (1943) stated that the chief means of spread was by infested cuttings, whose introduction into clean ground rendered the soil infective for at least 2 years. Moreton (private communication) stated that results of experiments suggested that the degree of infestation of chrysanthemum plants from the soil was small in land fallow during the winter, but

might be considerable if it was allowed to remain weedy until the spring. The object of this paper is to examine: (1) the importance of the soil as a source of infestation; (2) the extent of spread between plants, and (3) factors influencing the spread of infestation up the plant.

MATERIALS AND METHODS

Chrysanthemum eelworms used in the experiments were obtained from infested leaves, of various varieties, which were slowly dried and stored in cardboard boxes at room temperature. Eelworms were extracted by placing leaves under a mistifier in a Seinhorst's apparatus (Seinhorst, 1950).

To infest individual leaves with known numbers of eelworms, a piece of polythene sheeting 2×1 cm. was clipped to the undersurface of a leaf. Eelworms were transferred in a small drop of water to the space between the polythene and the leaf, then the whole plant was enclosed in a polythene bag. This method ensured that the small drop of eelworm suspension was distributed in a film which did not run off the leaf or dry up.

Leaf washings to remove eelworms present on growing plants were made as described by Wallace (1959*a*) and the technique used to determine the number of eelworms in a given volume of soil was similar to that used by Seinhorst (1956).

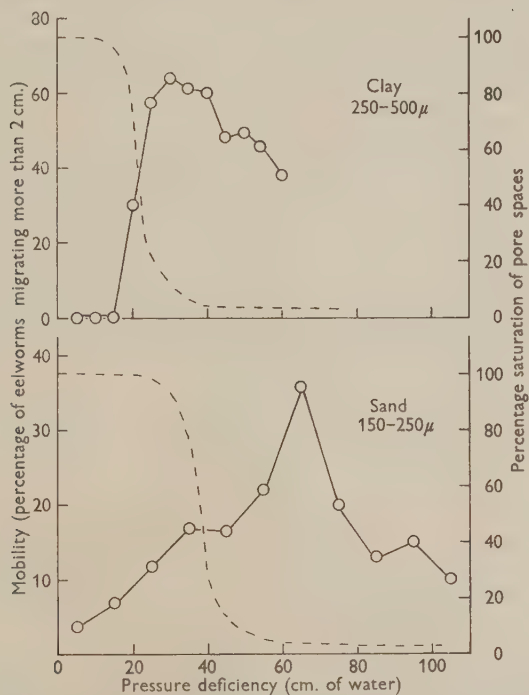
Methods used to study the mobility of chrysanthemum eelworm in sand of different particle size have been described previously (Wallace, 1958*a*).

RESULTS

There are three aspects to the problem of spread: (1) to what extent does the eelworm move from plant to plant during the growing season; (2) what factors determine the spread of infestation up the plant; and (3) how do new sources of infestation arise?

Active movement between plants may occur by migration through the soil or between leaves in contact. Passive migration may occur in moving water. Previous work on the movement of eelworms showed that mobility was affected by soil moisture content, soil particle size, eelworm length and eelworm activity (Wallace, 1958*b*). The chrysanthemum eelworm usually lives in the aerial parts of plants, and is highly active. Furthermore, it moves faster in thick water films than in thin ones while beet eelworm shows the opposite behaviour. The mobility of chrysanthemum eelworm in clay crumbs of particle size $250-500\mu$ and in sand of particle size $150-250\mu$ at different pressure deficiencies is shown in Text-fig. 1. In both media mobility rises with increasing pressure deficiency, it reaches a maximum when all the pores are emptied of water, and declines at higher pressure deficiencies. This response is similar to that of other eelworm species (Wallace, 1958*b*) and suggests that chrysanthemum eelworm is able to migrate through the soil. Molz (1909) and Marcinowski (1910) observed large numbers of this eelworm moving actively over leaves wetted by rain or mists. The lateral spread of eelworms among nursery-grown chrysanthemum plants was studied by taking soil samples and leaf washings in a plot of the variety Bill Speat adjacent to the variety Barry Meredith. At the beginning of July most of the Bill Speat plants showed clear symptoms of infestation but the Barry Meredith plants appeared to be uninfested. By

the end of September most of the Bill Speat and the adjacent Barry Meredith plants showed severe symptoms (Text-fig. 2). Three soil samples, each of five subsamples, and five leaf washings, each from four leaves, were taken at five different distances in each chrysanthemum variety; the results are given in Text-fig. 2. The highest concentration of eelworms in the soil and on the leaves is associated with the plants showing disease symptoms. The data suggest that there is little lateral spread through the soil or via the leaves. The chrysanthemums received overhead irrigation several times

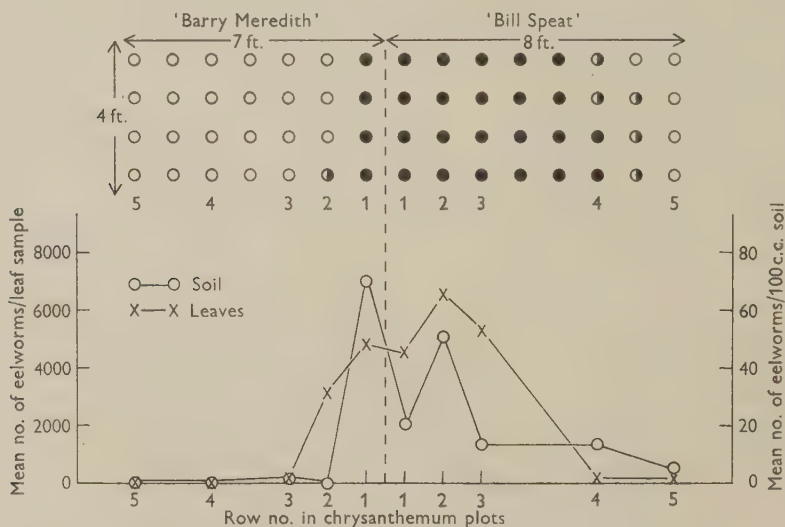


Text-fig. 1. Influence of pressure deficiency on the mobility of chrysanthemum eelworm in clay and sand. The dotted curves indicate the moisture characteristic. Each point is the mean of three replicates.

weekly so that lack of spread was probably not attributable to lack of moisture. Four other cases of apparent spread of eelworm from an infested variety to an adjacent uninfested variety were observed but the extent of the spread was never further than the second row of plants from the source of infestation, a maximum distance of about 30 in.

In plots of chrysanthemums at Rothamsted and at the National Chrysanthemum Society's variety trials at Bell Bar, Herts, sixteen varieties out of the 110 examined in the trials showed well-defined symptoms of eelworm infestation on 19 June 1959;

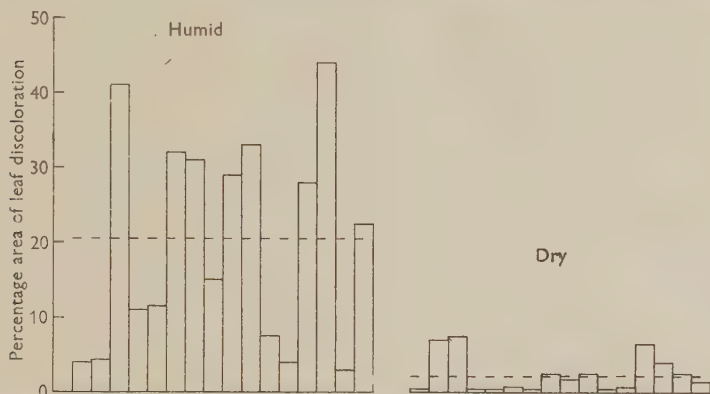
on 16 July no further varieties were found to be infested; on 17 August one further variety was recorded and on 28 September the total was fifty-six infested varieties. This sharp rise in September was accompanied by an increase in the general severity of eelworm symptoms within the infested varieties. Similar results were obtained at Rothamsted. It is suggested that the increase in rate of spread of eelworm infestation up the plants was related to the heavy mists which persisted through the night and sometimes for as long as 14 hr. Under these conditions the upper and lower surfaces of chrysanthemum leaves are covered with a layer of water droplets in which the eelworms emerge from the leaves and migrate up the plant (Wallace, 1959*a*). Thus, even in a dry year like 1959 severe chrysanthemum eelworm attacks can occur if there are heavy mists in the late summer.



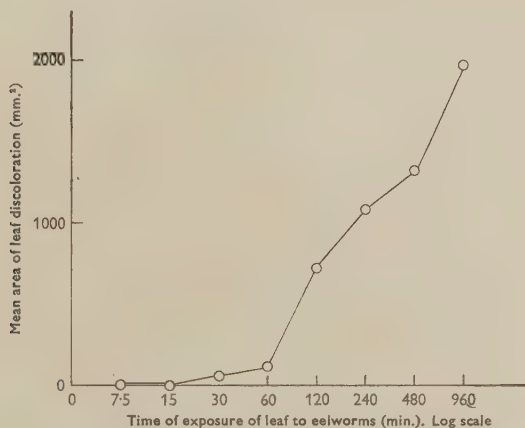
Text-fig. 2. Numbers of eelworms occurring in the soil and on the leaves of two varieties of chrysanthemums growing side by side. The plan of the two chrysanthemum plots is given above the graph; a black circle indicates a badly infested plant, half-black circle a moderate infestation and a white circle a healthy plant. The rows (1-5) in which samples were taken are also shown.

The influence of humidity on the extent of eelworm damage was examined in thirty-two isolated leaves of about equal size of the variety Westfield Bronze, each infested with 150 eelworms by the polythene strip method. The polythene was removed after 16 hr., and the leaves washed to detach any eelworms still on the surface. The leaf petioles were placed in sandy soil in a small pot embedded in damp peat in the laboratory. Sixteen of the leaves were entirely covered with polythene and sixteen were left uncovered. After 6 days, when symptoms of discoloration became visible, the total leaf area and the area of discoloration were measured (see Text-fig. 3). The

leaves under polythene remained covered with water drops throughout the experiment: the others were dry. The wet leaves showed a mean of 20% discoloured area, the dry leaves 2%; reflecting, it is suggested, the respective eelworm activities.



Text-fig. 3. Influence of humidity on the discoloration of artificially infested single chrysanthemum leaves. There were sixteen replicates for both the humid and dry conditions.



Text-fig. 4. Relationship between time of exposure of single chrysanthemum leaves to artificial infestations by the eelworm and the degree of discoloration subsequently produced. Each point is the mean of six replicates.

When an infested chrysanthemum leaf is wetted, the eelworms emerge, move over the leaves and up the stem, and then invade other leaves, the extension of infestation depending on the length of time that a plant remains wet. When the leaf is dry, movement and invasion cease. Wallace (1959*a*) showed that chrysanthemum eelworm can

move at least 6 in. up a stem overnight but it is not known how long it takes eelworms to invade a leaf. To study this problem 150 eelworms were held in contact with the undersurface of chrysanthemum leaves by the polythene strip method; after different lengths of time the leaves were well washed to remove any eelworms still on their surface. The leaves were then placed in soil in pots, covered with polythene as in the previous experiment, and after 6 days the areas of discoloration were measured. Text-fig. 4 shows that leaves exposed to eelworms for 7.5 and 15 min. showed no symptoms, but discoloration was present in leaves after 30 min. exposure, and thereafter the area of discoloration increased rapidly with increasing exposure time. These results help to explain why rapid spread of eelworm infestation up the plant occurs during the September mists. The importance of mists is also emphasized by Sanwal (1959) who used mist propagation frames to maintain populations of chrysanthemum eelworm for experimental purposes.

To examine the possibility that the soil was a source of eelworm infestation, experiments were made to study: (1) the ability of chrysanthemum eelworm to invade plants from the soil, (2) the extent to which a population of chrysanthemum eelworm decreases with time in soil under pot conditions, (3) the numbers of chrysanthemum eelworm present in soil of an infested plot during the summer, autumn and winter, (4) the eelworm infestation persisting in soil over the winter.

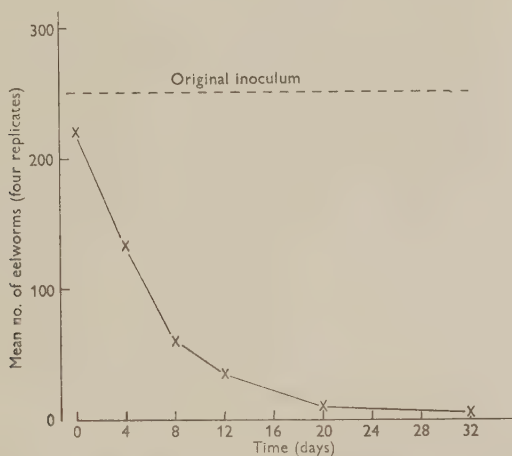
In the first experiment twenty-four cuttings were taken from warm-water-treated stools of variety Orange Peach Blossom, and after rooting were planted in pots of clean soil, where they were allowed to grow to a height of 1 ft. About 3000 chrysanthemum eelworms were then added to the soil about 1 in. from the base of the stem of each plant. Eighteen days later all these plants showed severe symptoms of eelworm infestation. Control plants (no eelworms inoculated) showed no symptoms. The plants were then removed and after a further 8 days half of the soil in each pot was examined for chrysanthemum eelworm by Seinhorst's sedimentation technique (Seinhorst, 1956). The number of eelworms recovered from any pot never exceeded five. Groundsel, a host of chrysanthemum eelworm, was sown in the remainder of the soil but showed no eelworm in the leaves after 10 weeks.

The problem of persistence of chrysanthemum eelworm in the soil was further studied in a pot experiment. Twenty-four small pots containing 100 c.c. of sterilized soil were well watered, allowed to drain, and then inoculated with 250 chrysanthemum eelworms. The pots were enclosed in polythene to prevent drying and four pots were taken at six time intervals over 32 days and the eelworms counted after extraction by the sedimentation method. The results, given in Text-fig. 5, indicate that the number of eelworms drops until, after 32 days, less than ten remain in the soil. This experiment suggests that chrysanthemum eelworm is probably unable to persist for more than 1-2 months in fallow soil, and it seems doubtful whether the eelworm can overwinter in the soil itself.

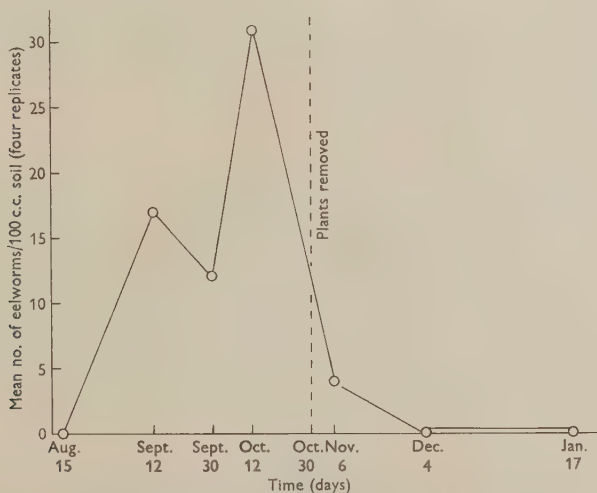
This result was substantiated by taking soil samples from August to January in an infested plot (Text-fig. 6). The level of the chrysanthemum eelworm population rose from zero on 15 August to a maximum on 12 October and coincided with the spread of infestation in the plants. Thereafter, the population decreased. The infested plants

were removed on 31 October and no *chrysanthemum eelworm* could be detected in the soil after the end of November.

In a final experiment to determine whether *chrysanthemum eelworm* could overwinter in soil, samples were collected from the worst infested plots in a nursery and



Text-fig. 5. Decline in population level of *chrysanthemum eelworm* in fallow soil in pots. Each point is the mean of four replicates. Horizontal dotted line indicates original number of eelworms inoculated into the soil.



Text-fig. 6. Soil population level of *chrysanthemum eelworm* in a heavily infested *chrysanthemum* plot from 15 August 1959 to 17 January 1960.

were stored outside throughout the winter 1958-59. Half of this soil was sterilized in March, and rooted cuttings from eelworm-free stools of variety Evelyn Bush were later potted on into the unsterilized and the sterilized soil. The plants were grown to maturity in pots but showed no eelworm symptoms, and leaves at the base of the plant failed to produce eelworms when placed in water.

These experiments strongly suggest that if land is clean-fallowed in the winter and spring, any infestation from the soil will be negligible; they also indicate that the chrysanthemum cuttings become infested from the parent stool. To test this, the stools of twelve lightly infested chrysanthemum plants, variety Lilac Nymph, were taken and dead leaves, green healthy leaves, stem cuttings, cuttings from runners (already rooted) and normal cuttings emerging from the base of the plant were removed and placed in water and/or stained with acid fuchsin/lactophenol. Adult and larval eelworms were found in all these plant parts except in the dead leaves, from which only adult eelworms emerged. The green healthy leaves yielded the highest numbers of eelworms; some cuttings seemed to be uninfested. A typical stool and some of the plant parts which were examined are shown in the Plate, figs. 1 and 2. The tip of a leaflet from the growing shoot of an infested cutting is shown in fig. 3: the nematodes here were living ectoparasitically within the curled leaflets. Nematodes were also found in the axils of the leaves and some had penetrated the stem tissue around the axial bud.

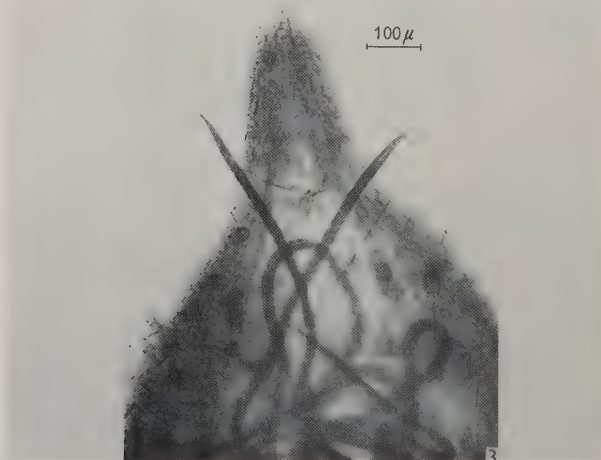
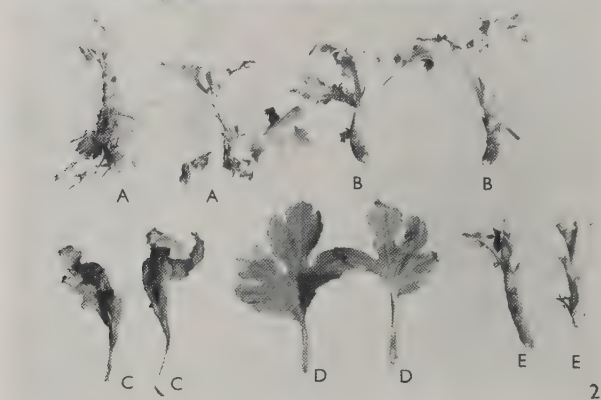
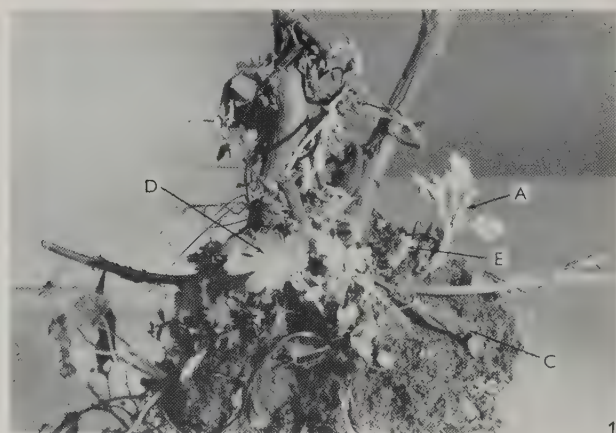
Further evidence of the importance of the chrysanthemum stool in maintaining the eelworm infestation arose during the routine work associated with the year's experiments. Stools of twenty-one varieties of chrysanthemum (some from very heavily infested stock) were warm-water treated; other stools were untreated. 313 cuttings from the treated stools were grown to provide a stock of healthy plants, and seventy-two cuttings from untreated plants were grown to provide a source of eelworms. At flowering time, five plants only from the treated stock showed eelworm symptoms, whereas all the plants from untreated stools were infested.

DISCUSSION

Data, from the experiments just described, indicate that clean fallowed soil is an unimportant source of infestation. The removal of weed hosts and treatment of the stool or cutting are therefore likely to provide more effective control than soil treatment with nematicides. The widespread eelworm infestations in England suggest that the present commonly used controls (parathion and warm water treatment) are not being used correctly. While chrysanthemum eelworm is killed by immersion in water at 115° F. for 5 min. (Staniland, 1950) there are practical difficulties in applying this treatment to chrysanthemum stools and an alternative treatment for stools is being sought.

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(See Paper II.)



EXPLANATION OF PLATE

Fig. 1. Chrysanthemum stool showing the plant as it is lifted in the autumn after flowering. Eelworms were found in all the labelled parts which are shown in detail in fig. 2.

Fig. 2. Component parts of the chrysanthemum stool shown in fig. 1. A, shoots arising from stolons; B, stem shoots; C, dead leaves; D, living green leaves; E, basal shoots—the type preferred by growers for use as cuttings.

Fig. 3. Tip of a leaflet from the growing tip of a basal shoot (E in fig. 2). Eelworms were found in large numbers, living ectoparasitically in the curled leaflet.

Observations on the biology of chrysanthemum eelworm
Aphelenchoides ritzema-bosi (Schwartz) Steiner
in florists' chrysanthemum

II. Symptoms of eelworm infestation

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(Received 16 May 1960)

SUMMARY

In stained sections of eelworm-infested chrysanthemum leaves the earliest effect of the attack is the appearance of a brown substance inside the leaf cells; this browning extends and ultimately reaches the leaf epidermis. The number of chloroplasts dwindles, the cell walls break down and large spaces appear in the leaf. Finally the leaf shrinks, the epidermis disintegrates and the brown substance is found on all the cell walls. The collenchyma around the main veins, where it reaches the upper and lower epidermis, does not break down until the final stage of attack.

Cuttings of thirteen chrysanthemum varieties, each artificially infested with about 110 eelworms, showed leaf blotches, stunting, blindness, browning of the stem and leaf petiole, leaf distortion, leaf scars or feeding areas and puffy, blistered and puckered leaves. Infested cuttings with severe symptoms may produce side-shoots or breaks which seem healthy but become badly infested later in the season when the plants are wetted by rain or prolonged mists. The eelworms often failed to penetrate further than the substomatal space in the youngest leaves. All the symptoms listed above, except leaf blotches, probably result from ectoparasitic feeding by the eelworm.

INTRODUCTION

Voss (1930), describing the symptoms of eelworm infestation in chrysanthemums, referred to leaves showing discoloured areas demarcated by the veins, and to injury to stems and blooms. Steiner & Buhrer (1933), Edwards (1934), Wilson (1943) and Goffart (1930) also described symptoms and the last-named gave coloured illustrations of infested leaves. Southey (1952) distinguished between damage to chrysanthemums caused by eelworms feeding ectoparasitically in the buds and endoparasitically in the lower leaves. There is little reference in these papers to eelworm symptoms in the chrysanthemum cutting or to the anatomy of the infested leaf—two aspects that are dealt with in this paper.

MATERIALS AND METHODS

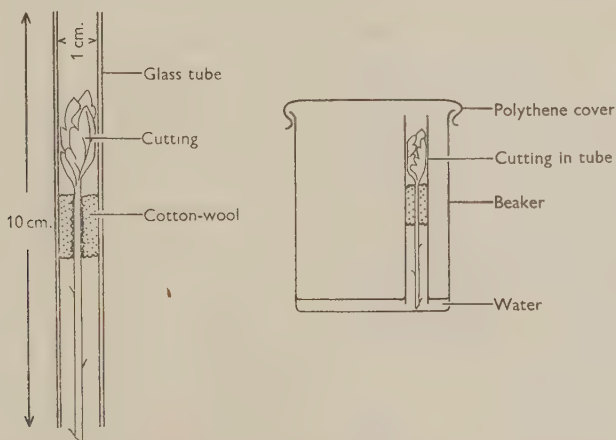
Leaf anatomy

Pieces of infested leaves were fixed in CRAF III (Sass, 1950), embedded in paraffin wax and microtomed. Sections were stained with fast green and mounted in canada balsam.

Quantitative infestation of chrysanthemum cuttings

It is essential to know the size of the initial eelworm infestation and to have uninfested controls to study eelworm pathogenicity and to compare the susceptibility of chrysanthemum varieties. As no quantitative method of artificially infesting chrysanthemum plants appears to have been published, except that described in part I of this investigation, four simple methods were devised and tested. About thirty eelworms in suspension were added to chrysanthemum plants in one of the following ways:

(1) Eelworms were absorbed on to soft tissue paper which was then wedged between the growing point and the leaf axil. This method was time-consuming and unsuccessful.



Text-fig. 1. Diagram of tube method for the artificial infestation of chrysanthemum cuttings with known numbers of chrysanthemum eelworm. Eelworms were added through the top of the tube to the leaves of the enclosed cutting. Inoculated cuttings in tubes were left in a closed beaker for 9 days and the cuttings were subsequently removed for rooting.

(2) Eelworms were placed on the undersurface of a leaf after it had been carefully wetted. This method is also impracticable; the leaves are difficult to wet because of leaf hairs and they are easily bruised. Only a few eelworms enter the leaves.

(3) Eelworms were pipetted on to the base of the stem. No infestation resulted probably because of the few eelworms used.

In the three methods listed above the inoculated plants were each enclosed in a polythene bag for 24 hr. to maintain a humid atmosphere.

(4) Unrooted cuttings were taken from eelworm-free chrysanthemum stools. An average of 30 or 153 eelworms was added in a drop of water to each of twenty cuttings each surrounded by a collar of cotton-wool and contained in a glass tube. The base of the cutting protruded from the tube and dipped into shallow water in a beaker which was covered with polythene (Text-fig. 1). Inoculated cuttings were left for 9 days,

when they were removed from the beaker. When the water in the beaker was examined, less than 0.6% of the inoculum number was found, indicating that the eelworms had remained on the cuttings. To find if the eelworms were still on the plant surface, each cutting and its cotton-wool were immersed in water in a specimen tube and after 24 hr. any eelworms in the tube were counted. The results indicated that an average of 86% of the eelworms entered the tissues, and this may be an underestimate because some worms probably emerged from the tissues into the water while soaking. To check the effect of the inoculation treatment, each cutting was dipped in hormone rooting powder and placed in sterilized sandy loam. After a further 8 days rooting commenced and the cuttings were lifted and stained with acid-fuchsin lactophenol. All were infested and it was clear that the eelworms had multiplied considerably.

RESULTS

Symptoms of eelworm infestation on cuttings

Cuttings of thirteen varieties of chrysanthemum were each infested by the tube method with an average of 114 eelworms. The following clearly recognizable symptoms appeared in the cuttings when they had rooted and were growing on:

Discoloured leaves. Lower leaves on cuttings often show browning between the veins resembling the leaf blotch of mature plants. Initially, the browning may take the form of minute spots which increase in area and coalesce to fill the interveinal zone. Stained preparations of young chrysanthemum leaves in the early stages of infestation frequently show an eelworm entering the leaf through a stoma. The cells adjacent to the stoma are often brown and it appears that, in young leaves, the eelworm is confined to substomatal space, where it feeds, producing the brown spots. The intercellular spaces in young leaves are very small and would restrict eelworm movement; in older leaves they are large enough to allow eelworms to move freely through the tissues.

Reduction in height. Severely infested cuttings are often stunted because of damage to the growing point, often producing an abnormally bushy type of plant with short internodes.

Blindness. This is a common symptom. The shoot does not grow and turns brown. Blinded plants may throw premature side-shoots or breaks which are lower on the plant than those obtained by stopping, i.e. pinching out the growing shoot.

Brown stem. Browning of the stem and petioles close to infested leaves occurs often in the form of ladder-like scars composed of a scurf of dead cells. These scars appear to be the result of eelworms feeding externally on tissues while still in the bud. The ladder-like appearance is due to the brown patch of cells extending as the plant grows and splitting at right angles to the principal direction of growth.

Distortion. Leaves may be asymmetrical, or simply spatulate or linear.

Needling. An extreme symptom of distortion where the leaves are long and needle-like. Such leaves may be produced, in the absence of eelworms, on the earliest cuttings of some varieties.

Feeding areas. Brown scars are visible on the leaf surface. These scars are similar to

those on stems and petioles but do not form a ladder-like pattern, probably because growth is similar in each dimension of a leaf.

Puffiness and blistering. The surface of the leaf forms small blisters and crinkles. In some cases almost all the leaf has a puffy, fleshy appearance.

These symptoms are shown in Pl. 1.

Cuttings of different varieties tend to show specific syndromes of infestation; this aspect will be dealt with in a later paper. Cuttings showing marked symptoms often produce side-shoots or breaks which at first appear to be free from eelworm and grow vigorously. Such plants, however, may show eelworm symptoms at a later stage when the plants are wetted for long periods by rain, mist or dew.

Anatomy of infested leaves

Leaves showing different degrees of infestation were taken from several chrysanthemum varieties and processed as described earlier in the paper. Examination of a series of transverse sections of such leaves shows that in the initial stage of infestation, eelworms may occur in the mesophyll without causing any apparent symptoms. The first reaction by the plant is the production of patches of an opaque brown substance within the cells near the eelworms (stage 1). This browning becomes more extensive and the number of chloroplasts within the cells is reduced (stage 2). Browning eventually extends to the epidermis, few chloroplasts now remain, and cells in the mesophyll are ruptured (stage 3). The leaf cells eventually become almost totally brown with no chloroplasts, cell walls break down and large spaces appear in the mesophyll (stage 4). In the final stage the leaf is shrunken, the disintegrated cells have a thick layer of brown substance on their walls and the epidermis is broken down (stage 5).

During the process of necrosis the mesophyll and palisade tissue are equally affected and, although most eelworms occur in the air-spaces in the mesophyll, some penetrate between the palisade cells. The symptom of brown areas of the leaf demarcated by veins is shown well in the transverse sections (Pl. 2). In the large veins the chlorophyll-free collenchymatous cells of the vein sheath extend from the upper to the lower surface and thus bar any extension of leaf necrosis, but in small veins the collenchyma does not reach the epidermis and the stele is often surrounded by necrotic cells. In late stages of leaf necrosis even the collenchyma breaks down and infestation is able to spread across the large veins. Large spaces containing eelworms often appear in the mesophyll close to the vein and in many leaves in the final stages of cell degradation, eelworms are still found in large numbers.

DISCUSSION

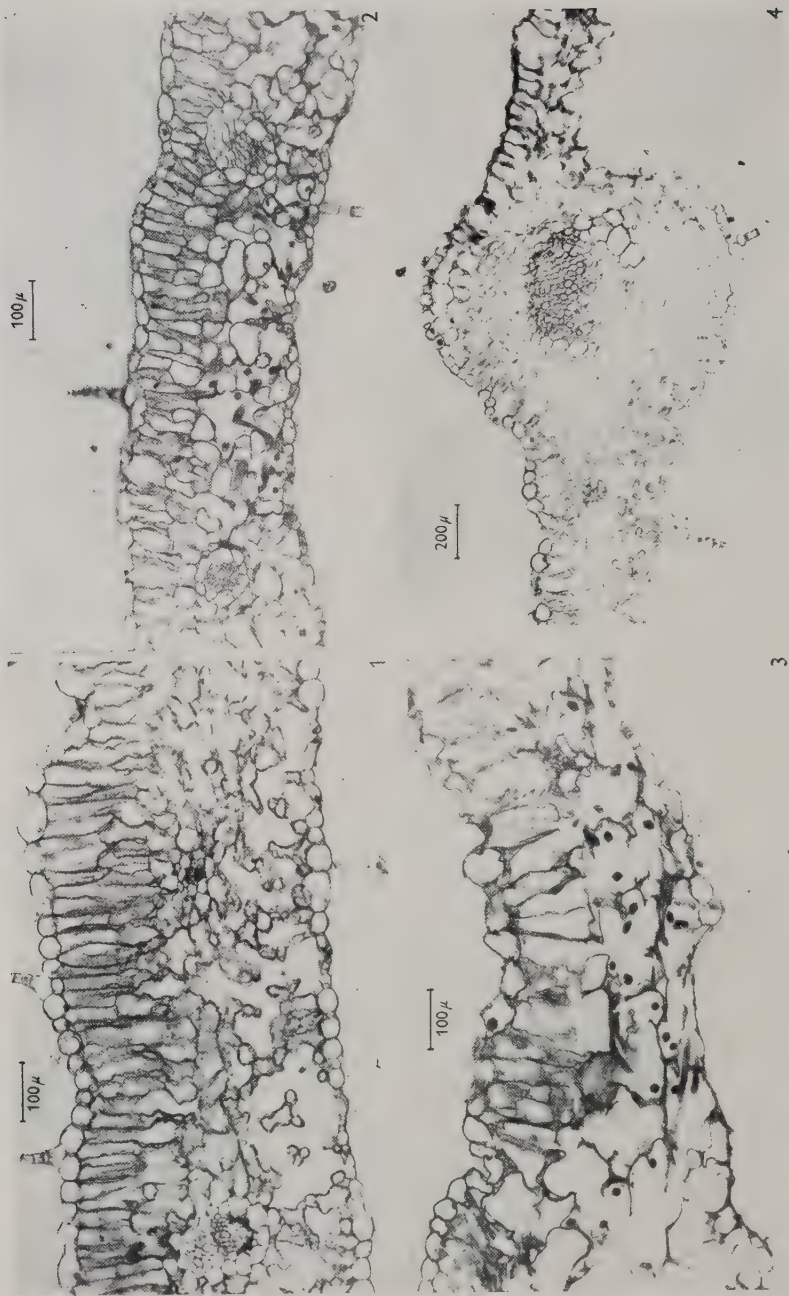
The formation of a brown substance in chrysanthemum leaf cells infested with eelworm may be due to oxidation of polyphenols to quinones by the enzyme polyphenol oxidase (cf. Byrde, 1957) followed by polymerization to the brown substances; although the enzyme and its polyphenol substrate may occur together in the same cell, they are separated in the healthy leaf. Whether the eelworm acts solely as a macerating agent allowing enzyme and substrate to meet or whether it contributes an enzyme of its own

remains to be investigated. The disappearance of chloroplasts from the cells is a common reaction of leaves to pathogens and is usually associated with loss of protein (Bonner, 1950). The role of the eelworm in this process is also unknown. The fact that the collenchyma is not degraded until a later stage suggests that these cells have different properties from the mesophyll and palisade cells. The lower and more mature leaves of the chrysanthemum plant are usually those in which leaf blotches are seen, and this has, perhaps, given undue importance to the opinion that the plants are infested from the soil. On cuttings, infested by the tube method, leaf blotches also occur only on the lowest leaves, although eelworms may be found in all the green tissues irrespective of their age or position. It seems that the youngest chrysanthemum leaves are probably physically and chemically different from larger leaves, and the eelworm symptoms are correspondingly different. A physical and chemical study of chrysanthemum leaves of different age and from several varieties is in progress.

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EXPLANATION OF PLATES

PLATE I

- Fig. 1. Typical leaf blotch symptoms due to internal feeding of chrysanthemum eelworm.
Fig. 2. Distorted leaves, blind terminal bud and stem scars due to external feeding of chrysanthemum eelworm on a rooted cutting.
Fig. 3. Brown stem, dwarfing (short internodes), distorted leaflets and damaged terminal bud of a rooted cutting infested with chrysanthemum eelworm.
Fig. 4. Stem scars, puckered leaf (top right hand) and 'needle leaf' caused by the eelworm infestation of a young chrysanthemum plant.

PLATE 2

- Fig. 1. Transverse section of an uninfested chrysanthemum leaf showing chloroplasts in mesophyll and palisade tissue cells.
Fig. 2. Transverse section of a chrysanthemum leaf—early stage of infestation. Eelworms in transverse section can be seen in mesophyll air-spaces; there is a loss of chloroplasts from the mesophyll cells (cf. fig. 1) and some formation of a brown substance on cell walls.
Fig. 3. Transverse section of chrysanthemum leaf—late stage of infestation. Eelworms in transverse section are visible in the mesophyll air-spaces and chloroplasts are absent. There is considerable disintegration and extensive browning of the cell walls.
Fig. 4. Transverse section of infested chrysanthemum leaves. Symptoms apparent on one side of leaf vein only, demonstrating the efficacy of the leaf vein as a barrier to spread of the eelworm inside the leaf.

Naming of some strains of soft-rot coliform bacteria—a correction

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(Received 12 September 1960)

In recent papers in this *Journal*—‘The coliform bacteria associated with potato black-leg and other soft-rots’ (48, 51–57, 58–64) by two of us (D.C.G. and W.J.D.), it is stated in table 1 on page 54 and also on page 62 that the soft-rot bacteria attacking *Philodendron*, *Parthenium*, *Chrysanthemum* and *Dianthus* (carnation) should be considered as one variety of *Pectobacterium carotovorum* and named *P. carotovorum* var. *chrysanthemi*.

This led to some correspondence in which one of us (E.H.) pointed out that Starr (1947, p. 299) had described the *Parthenium* pathogen as *Erwinia carotovora* f.sp. *parthenii*, which had been altered to *Pectobacterium parthenii* by Hellmers (1958, p. 128). In the same account Hellmers altered *Erwinia chrysanthemi* Burkholder, McFadden & Dimock (1953, p. 526) to *P. parthenii* var. *chrysanthemi* (Hellmers 1958, p. 129) and also created *P. parthenii* var. *dianthicola* (Hellmers 1958, p. 129), thus raising the name *parthenii* to specific rank. Hellmers (1958) also suggested gathering the three organisms into one stirps (or variety) named *stirps* (var.) *parthenii*.

The recent investigations reported by D.C.G. and W.J.D. in this *Journal* have further confirmed this point of view, but we now prefer to consider the three organisms concerned, along with the *Philodendron* bacterium, as a single *variety*. According to the International Code of Nomenclature of Bacteria and Viruses, 1958, Rule 22c and Principle 9, priority for a name is invariably given to the oldest, which in the present instance is *parthenii*. Hence the correct name for the organisms concerned is *Pectobacterium carotovorum* var. *parthenii* (Starr) comb. nov.

Emended diagnosis. *Pectobacterium carotovorum* var. *parthenii* deviates from *P. carotovorum* (Jones) Waldee, and *P. carotovorum* var. *atrosepticum* (van Hall) Dowson through its slow fermentation (after 9–20 days) of lactose, positive test for indole, pink medium and colonies on Endo-agar, pink or yellowish colonies on deoxycholate-agar, alkaline reaction in sodium malonate and sodium acetate, and it cannot produce black-leg in potato plants at temperatures lower than 19° C.

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Proceedings of the Association of Applied Biologists

Meetings of the Association were held on 27 May, 22–23 September, 14 October, 11 November and 9 December. The full list of speakers and papers at the 1960 Meetings will be given in the Report of the Council.

Abridged versions of the following papers are presented below:

Meeting of 22 September

Aspects of black currant gall mite infestations. By Mr C. A. COLLINGWOOD and Miss A. M. BROCK

Meeting of 14 October

Metabolism of soil invertebrates in relation to soil fertility. By Mr AMYAN MACFADYEN.

Ann. appl. Biol. (1961), **49**, 211–215

Aspects of black currant gall mite infestations

By C. A. COLLINGWOOD

N.A.A.S., South-west Region

AND A. M. BROCK

N.A.A.S., East Midland Region

This paper discusses the practical implications of two aspects of gall mite infestation. The first observation relates to mite invasion of fresh buds in spring. In an earlier paper (Collingwood & Brock, 1959) we showed empirically by infestation experiments that maximum mite transference to clean plants occurred just after full blossom. This was elaborated and confirmed by series of spraying trials described by Collingwood & Dicker (1960) in which single endrin sprays were shown to have maximum effect when applied at early fruit set.

The observations described here result from the examination of shoots and direct mite counts in new buds from early grape stage to fruit swell. Results shown in Fig. 1 demonstrate that not much activity occurs until blossom and confirm that the greatest invasion of mites takes place between blossom and setting. It may also be seen that routine lime sulphur applications at grape stage would normally precede maximum mite movement by about 3 weeks by which time much fresh extension growth would occur and little sulphur deposit remain. It is clear that pre-blossom miticidal sprays should be applied as near to blossom as possible as has already been shown empirically in the spraying trials referred to above.

The general picture suggested in Fig. 1 will be modified at times at different sites. Thus in 1957 heavy invasion of buds took place at Norton Disney, Lincs, a fortnight earlier than at Dowsby on more vigorous softer growing bushes in the same county. This year at Sutton Bonington large numbers of mites—up to 25 in some buds—were recorded before the end of April, at early blossom. These variations of timing at different sites and seasons underline the need for a programme of at least two miticidal sprays to ensure good control, but the observations also confirm that the programme—first open flower and 3 weeks later—that was developed

empirically for co-operative spraying trials in the N.A.A.S. as a standard procedure (Collingwood, Vernon & Legowski, 1960) will effectively cover the period of maximum mite activity and at the same time allow for seasonal differences in the large majority of plantations.

In some plantations, especially in areas of high rainfall and soft growth, a small proportion of over-wintered galls will continue to remain green and retain a viable population of mites until August or even later. Such old galls continue to release mites in small numbers over a long period. These mites appear to exhibit migratory movements as described by Smith (1960). Such mites may constitute a danger to nearby nursery beds which have been cut down in spring and have made late growth, but normally new buds are sufficiently developed and tight by early June to be no longer penetrable by later migrating mites.

The second aspect now considered is the effect of gall mite infestation on yield.

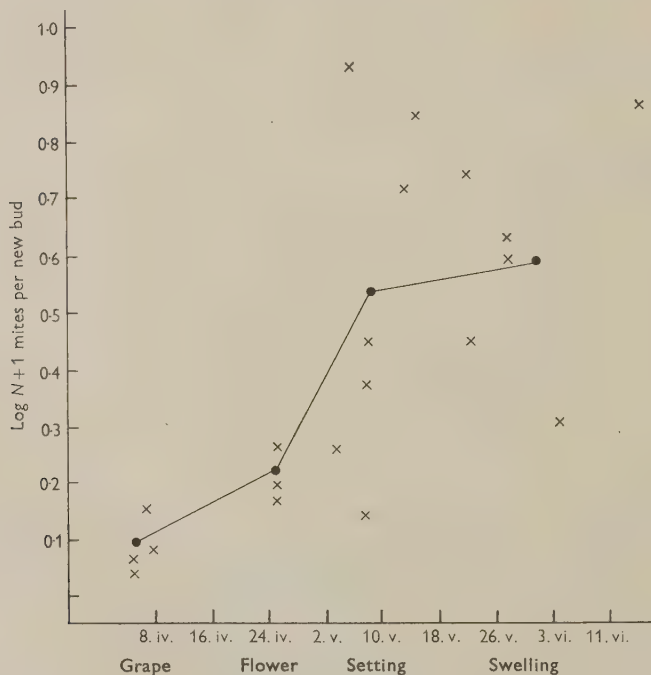


Fig. 1. Mite invasion of buds.

Table 1. *Crop yield and infestation level*

	No. treated	Endrin at		Standard error
		Grape	Set	
% galls Nov. 1957	15	25	13	—
% galls Nov. 1958	37	18	1	—
% galls Nov. 1959	41	18	1	—
1959				
Mean length new wood, in.	10	11	13	0.75
Blossom trusses per shoot	9	20	34	3.3
Crop yield per 4-bush plot (oz.)	29	47	51	11.6

In spraying trials at Sutton Bonington, infestations have been assessed by estimating the percentage of galls on each plot in the autumn of each season. Certain plots had had the same treatment over two seasons, and some of these were harvested in 1959, representing three levels of infestation over the two years.

Enhanced growth appeared in 1958 on plots where the mite had been well controlled, but shoot measurements showed no significant differences in that year. By 1959, after two seasons of spraying, tip growth was significantly longer on the low infestation plots than on the untreated controls at $P = 0.01$. At the same time there was a marked increase in the number of blossom trusses on the endrin-treated plots as shown in Table 1. Both growth effects and increase of blossom were directly attributable to the suppression of mite damage to the buds on the treated plots. When plot yields were compared, however, although there was a definite trend for the lower yields to occur on the most heavily infested plots with a significant negative

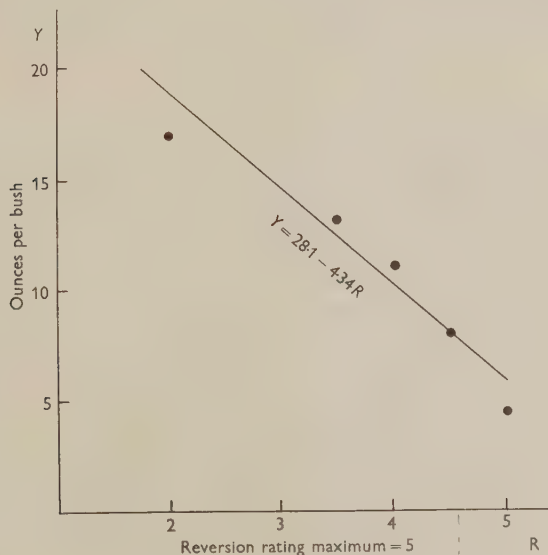


Fig. 2. Reversion and crop yield 1959.

correlation ($r = -0.517$), all plot yields were low and not significantly different according to spray treatments.

It became evident while picking these bushes that the low yields were associated with failure of the blossom to set, and this was attributed to reversion. All the bushes on the experimental area were thereupon scored for severity of reversion symptoms. They were all found to be more or less severely reverted according to the accepted leaf character symptoms as described by Lees (1922). The intensity of reversion symptoms according to the scoring system adopted was found to be more significantly and positively correlated to the intensity of infestation in 1957 on each of the bushes examined ($r = 0.499$, $n - 2 = 30$) than to the intensity of infestation in 1958 ($r = 0.318$, $n - 2 = 38$).

This suggests that reversion spread through the plantation probably as a result of mite infestation during 1957 or even earlier. The application of endrin in 1958 and 1959 markedly reduced mite infestation on certain plots. This was accompanied by increased growth and bloom which did not, however, apparently offset the effects of reversion. There was in fact a

higher negative correlation between yield and reversion ($r = -0.549$, $n-2 = 13$) than between yield and mite infestation ($r = -0.517$, $n-2 = 13$). Fig. 2 shows the regression of yield (Y) on reversion (R) with the calculated equation, $Y = 28.1 - 4.34R$. According to this, by extrapolation with no reversion, the plantation would have cropped at slightly over a ton/acre which might correspond fairly well to expectation with a 13-year-old neglected plantation in a drought year of poor set. In fact the mean plot yield in 1959 was $\frac{1}{3}$ ton/acre and the plantation had evidently become so diseased as to be economically useless.

In Lincolnshire, individual bushes in one plantation had been tagged by the grower for signs of incipient reversion in the spring of 1956. These bushes were found to have a substantial gall-mite infestation corresponding to a general level of about 12 % of galled buds. Infestation and the early symptoms of reversion, including high colour and lack of bloom of the calyx, were often very localized together on the bush. By 1959 most of the tagged bushes were in the last stages of reversion and had virtually ceased to crop. There was a close agreement in this plantation, as also in a 6-year-old plantation at Sutton Bonington, between the contour of mite infestation and reversion, and at both these sites experimental spraying had failed to rehabilitate the bushes despite achieving a marked reduction of galling in certain areas.

DISCUSSION

Massee (1952) gave evidence to show that reversion was transmitted by gall mite and the close association of the two has been generally accepted since Lees (1917) first drew attention to their possible connexion. The observations described above provide additional circumstantial evidence to support the close connexion between them although the evidence for the actual transmission of reversion by the mite provided by Massee (1952) is open to criticism. An important difficulty preventing the clear-cut demonstration of transmission is that recognition of reversion is at present based solely on symptoms and not on the more objective tests that would be provided by chemical methods or by transmission to quick-reacting test plants. It is not therefore possible in the present state of knowledge to assume that normal-looking bushes are in fact free from the disease. However, general field experience and circumstantial evidence of the kind described above confirms the close link between gall mite and reversion and this raises certain considerations with regard to management and chemical control.

It would appear from the observations discussed above that, once bushes have become infested to the extent of 12 % or more galled buds, there can be no prospect of rehabilitation through chemical spraying and that cropping is likely to decline to low uneconomic levels after about two seasons through the irreversible development of reversion. Blocks of such bushes are best grubbed. Frequently, however, areas of severe mite infestation are patchily distributed within an otherwise economic plantation. In such cases chemical control to prevent the mite infestation from spreading rapidly throughout the cropping area becomes imperative. Work on chemical control reported elsewhere (Collingwood & Dicker, 1960; Collingwood, *et al.* 1960) has shown that in general, traditional applications of lime sulphur will not prevent the build up of infestation. Existing infestations will be held at about the same level where at least two or more applications of lime sulphur are given or where endrin is applied just before blossom. Post-blossom sprays of either endrin or fluoroacetamide are necessary to reduce the level of infestation. Since difficulties arise with the use of these chemicals after blossom on fruiting plantations, it is of greatest importance to concentrate on the early health of a plantation by rigorous roguing and the thorough spraying of stock beds, cuttings and transplants.

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Ann. appl. Biol. (1961), 49, 215-218

Metabolism of soil invertebrates in relation to soil fertility

BY AMYAN MACFADYEN

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It is useful, if not quite accurate, to distinguish between the mechanical and chemical activities of the soil fauna. Other papers have been concerned with mechanical activities and I do not propose to consider them now except to point out that in most fertile soils the greater part of dead plant matter is processed by the guts of the larger invertebrates. When we consider moorland and other poor soils, however, these larger forms are usually the first to disappear—with marked effects on the structure and constitution of the soil.

I propose to consider the chemical effects of the fauna under two headings: first, effects resulting directly from the animals' metabolism and secondly, those in which the animals' role is more in the nature of a catalytic one.

The difficulty of comprehending the interacting effects of animal metabolism on soil can be appreciated when it is remembered that most soils contain up to 1000 species of animals. The complexity of the 'food web' in such a situation is enormous and the best way to start to study such a system is to make measurements and try to ascertain which of many possible links are the most important.

Unfortunately this is not yet possible for any single environment. However, I have, by taking many liberties with the data and combining figures from a variety of sources, produced a diagram (Fig. 1) which illustrates approximately the way in which the energy of photosynthesis is transferred through a typical meadow under temperate conditions. Only about 0.5 % of solar energy striking the earth's surface is utilized by photosynthesis and the 'channels' on the diagram (whose width is logarithmically proportional to the energy flow in small calories per square metre per day) illustrate the fate of the photosynthesized energy.

The main points to notice are that about one-sixth of the whole is immediately dissipated by plant respiration, that although one-quarter is consumed by herbivores about half this is returned to the soil as faeces, dead bodies and other waste products, so that finally nearly three-quarters of the whole amount passes to the soil as dead organic matter. In other words when we exploit pasture by cattle grazing less than one-sixteenth of the products of photosynthesis is potentially available to the cattle and less than 0.05 % is in fact likely to contribute to human food.

The 'boxes' representing the calorific content of the standing crops of the different groups of organisms are also drawn on a logarithmic scale. It should be noticed that the arbitrarily fixed ratio of standing crop to respiration per day decreases through the food chain; it requires about 1000 calories of plant protoplasm to process 1 calorie per day in respiration but the ratio for cattle and for man are about 200 and 150 to 1.

The second figure (Fig. 2) shows what happens to the energy reaching the soil as organic waste. Here data are even more difficult to come by and I have not even managed to complete the picture; especially since the relations of the predators to their prey are uncertain and the proportion of energy metabolized to food attacked is rarely known. I have therefore given only

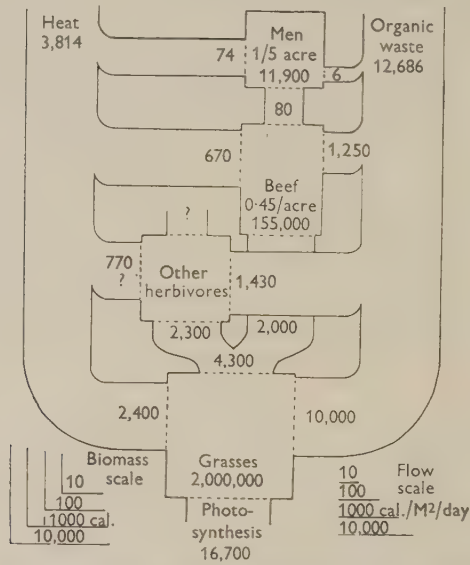


Fig. 1. Transfer of energy of photosynthesis through a typical meadow.

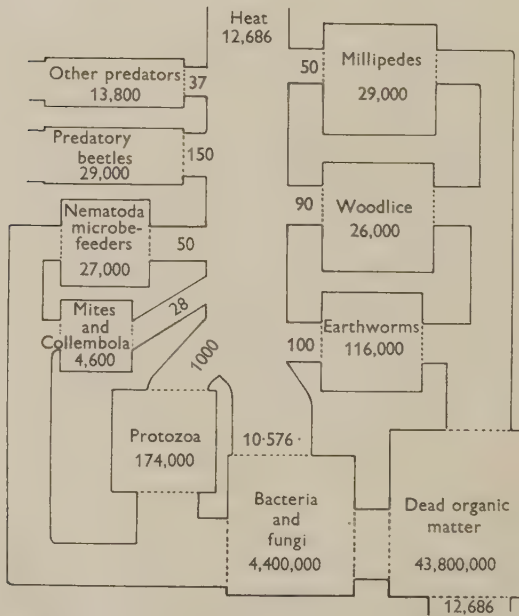


Fig. 2. Fate of energy reaching soil as organic wastes.

net energy utilization figures for these organisms and it should be appreciated that much of the food 'consumed' is returned to the reserve of dead organic matter as material killed but not eaten and as faeces.

It will be seen from the flow figures that the microbes liberate about 85 % of the energy, protozoa perhaps 8 %—although this is a particularly variable and unsatisfactory figure—the larger invertebrates about 4 % and the small arthropods and nematodes about 4 % between them. The ratio of standing crop to flow is about 400:1 for the micro-organisms, 200:1 for the 'meso fauna', and varies up to nearly 1000:1 for the large invertebrates.

Looked at from the point of view of the plants, dead plant matter once used contains valuable nutrients now out of circulation and the longer these remain bound up the less available will the nutrients be. Thus 'bottle-necks' in the energy-flow picture represent a threat to fertility, while anything which increases the flow from the large 'boxes' in the diagram—the reserves of dead organic matter and to a lesser extent the micro-organisms and large invertebrates—will increase the availability of nutrients.

If we are to understand more of the role of animals in clearing these bottle-necks we need to know more about the numbers and food preferences of the fauna and microflora, we require metabolic life tables of the different species showing how much whole populations respire, we need knowledge such as Phillipson (1960) has recently provided for *Mitopus morio* on the relations between feeding and metabolism and we also require much more information on particular soil types, especially the poorer ones.

THE CATALYTIC ACTION OF ANIMALS ON SOIL METABOLISM

It is only recently that we have begun to realize that animals may have effects on the metabolism of the soil out of proportion to their own respiration rates. This conclusion is to some extent conjectural and I should like first to list a number of observations and facts which point towards it.

(1) The numbers of living micro-organisms visible in soil are usually low in comparison with those which become apparent when small pieces of humic material are incubated in humid conditions in the absence of animals.

(2) The census of soil fauna shows that there are numbers of the order of 10^5 microbe-feeding arthropods and 10^6 to 10^7 microbe-feeding nematodes per square metre. Rough calculations based on Witkamp's (1960) work indicate that the arthropods may consume up to ten times their respiration value of such forms—in other words, that they are inefficient but prolific browsers of micro-organisms.

(3) Hinshelwood (1951) was perhaps the first to show that bacterial colonies, after initial phases of growth and peak activity, become senescent. This has since been confirmed and also shown to apply to fungi. This means that micro-organisms in the absence of grazing become 'bottle-necks' in which metabolism is slow in relation to energy content and it suggests that grazing by animals may accelerate the energy flow.

(4) The widespread occurrence of mycostasis and bacteriostasis resulting—it is suggested—from the presence of antibiotic substances in soil means that much potential microbial activity is held in abeyance. Stasis of this kind can be abolished by some plating techniques used to estimate micro-organisms. It has also been shown recently (Witkamp, 1960) that the introduction of single terrestrial Trichoptera or *Glomeris marginata* to soil cultures can cause hundred-fold increases in spore germination.

The doubling of total soil respiration by the introduction of small numbers of earthworms reported by Satchell (1960) possibly illustrates a different aspect of the same phenomenon.

(5) The demonstration by Witkamp (1960) that soil oribatid mites can rapidly cause the re-infection of soil samples by transporting micro-organisms on and inside their bodies illustrates another way in which microbial metabolism may be enhanced due to insemination in more favourable places.

(6) It seems likely that the corpses, faeces and exuviae of animals will provide enriched sites from which soil micro-organisms can spread into surrounding soil. Satchell (1960) has given a figure of 2 cwt. per acre as the equivalent in sodium nitrate to the nitrogen content of earth-

worms. The small forms often have very short generation times—a few days for the smallest nematodes, a few weeks for many arthropods—and must produce great numbers of corpses containing high concentration of nutrients. Some fungi, of course, do not wait for the deaths of their food organisms and capture them alive.

These points seem to me to show that we should take seriously the possibility that soil animals promote metabolism on a vastly greater scale than that which results from their own internal physiology: by grazing, by breakdown of 'stasis', by transport of spores and by producing foci of high nutrient status. This working hypothesis is open to investigation by simple laboratory experiments, by direct observation, by exclusion experiments such as those pioneered by Bocock & Gilbert (1957) and by the measurement of respiration rates of systems of varying complexity. I would advocate this as a field of research in which the problem can now be defined, the techniques exist, the theoretical interest is great and the practical significance with respect to our understanding of the measuring and control of fertility cannot be doubted.

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Reviews

An Introduction to Bacterial Physiology. By EVELYN L. OGINSKY and W. W. UMBREIT.
Pp. 443. Second Edition. W. H. Freeman and Co., San Francisco. 1959.
\$7.50 (£3. 4s.).

The authors of this work state in their preface that as 'we feel that bacterial physiology is the basis of bacteriology we have designed this text-book for a course at the undergraduate level—perhaps immediately following the course in general bacteriology, perhaps postponed until the senior year'. This possibly illustrates a difference between the American presentation of a subject for undergraduates and that adopted by most British universities.

The book consists of nineteen chapters grouped under six Sections, namely: the nature of bacterial physiology, bacterial anatomy, populations, metabolism, variation, and the capacity of the cell. These sectional headings will give some idea of the scope of the work. At the end of every chapter is a useful list of up-to-date references followed by some thought-provoking 'questions'.

The book is well printed on glazed paper and is written in a clear style accompanied by some excellent reproductions of electron micrographs. In addition, there are many intriguing black and white line diagrams which certainly catch the eye and necessitate some hard thinking to discover what they are all about. As an example, that on p. 22—'Imaginary Representation of a Small Particle Approaching a Bacterial Cell'—might, at first glance, be taken for a medieval sea fight. They may appeal, however, to those who think in pictures and regard particles and molecules as *solid* structures. A stimulating book.

W. J. DOWSON

The Cell Nucleus. Edited by J. S. MITCHELL. London: Butterworths. 1960. 50s.

Once upon a time we were assured that pentose nucleic acid was characteristic of plants and deoxypentose nucleic acid of animals; a little later the idea was that RNA was cytoplasmic and DNA nuclear; now the DNA is supposed to carry instructions into a cell and hold them there while RNA transfers them to enzymes. Will this turn out to be a fairy tale too? Probably not if the proposition is amended by the insertion of the word *some* six times. Thus amended it becomes less susceptible to disproof; equally it becomes less useful as a guide to experiment. At present the proposition is acting as a powerful stimulus for research, and it was referred to, though not always sympathetically, by most of the participants in this symposium organized by the Faraday Society in autumn 1960.

Ultimately, Biochemistry will probably develop methods for studying the simultaneous metabolism of many different substrates by many different enzymes in unfractionated systems. These methods will be more relevant to the *in vivo* working of a cell than our current studies on isolated or fractionated systems; studies that lead some biologists to relabel our activities Thanatochemistry. But that time has not yet come and one reads this symposium with some exasperation that so much effort should have been lavished, and so many confident statements based, on such dubious techniques. Clearly effort of this type is an essential step towards better methods; criticism would be silenced if the conclusions were more tentative.

The nucleic acids are the centre about which discussion turns. They are often treated as if they were definite entities so that it would be meaningful to discuss the metabolism of RNA in, for example, a certain nucleolus. There are two circumstances in which that would be permissible: if all RNA particles were identical, as molecules of ATP are, though doing different things in different places; or if they differed slightly, as insulins do, while all acting similarly. But according to the 'information theory' picture of nucleic acid action, neither of these possibilities is comparable to the actual position. Instead we have an immense number of different structures, linked together perhaps, doing different things at different sites. Logically, it would be wasteful

to have more than two of any one type in the same cell. Thirty years ago, when we thought of nucleic acid metabolism primarily in relation to gout, it was as reasonable to talk of nucleic acid metabolism as it now is to talk of glycogen metabolism. But not now. The analogy is now with protein metabolism. There is no more reason to assume that the different forms of DNA are metabolized similarly than to assume that oxytocin, pepsin, haemoglobin and collagen are.

For all that, there is much valuable information (along with more speculation and still more disagreement) in this symposium. The effects of radiation on the whole animal, on tissue preparations, and on tissue components *in vitro*, are dealt with by several participants. Mirsky makes some good points about the movement of enzymes into and out from the nucleus during processes of fractionation. Dounce, Barnard, Kirby, and others discuss the nature of the bonds holding nucleoproteins together; the conflict here suggests that a symposium on the linkages uniting proteins to prosthetic groups would be timely and useful.

The editor and publisher may be congratulated on getting this record out in less than a year from the time of the meeting. They deserve less praise, at a time when many publishers are at last yielding to the arguments many of us have been putting forward for 20 years and are producing paper-backs, for the fancy and expensive format they have given an ephemeral work. There are too many misprints, and most readers will be astounded to find phenylalanine hyphenated like that.

N. W. PIRIE

Behaviour and Evolution. Edited by ANNE ROE and GEORGE GAYLORD SIMPSON. Pp. 557. New Haven: Yale University Press; London: Oxford University Press. 1958. 80s.

This volume, the result of two conferences sponsored by the American Psychological Association and the Society for the Study of Evolution, forms an important attempt to bridge the gaps which exist between many students of evolution and those concerned with animal behaviour. A considerable amount of thought and planning has gone into this volume. In fact, the method of presentation which has been adopted should be considered by all editors of future symposium volumes. The first conference explored possible fields of co-operation and at the second conference pre-circulated papers were presented in summary form and most of the time was devoted to a general discussion of their contents. After this meeting the papers were then rewritten in the light of the discussions which had been held. In this volume we are spared the undigested and laboured discussions which so often clutter up books of this kind. The principal sections of the book are: The Study of Evolution and its Record; The Physical Basis of Behaviour; Categories of Behaviour; The Place of Behaviour in the Study of Evolution; Evolution and Human Behaviour.

The twenty-six contributors to the volume include, in addition to the editors: Marston Bates, F. A. Beach, T. H. Bullock, A. E. Emerson, Julian Huxley, E. Mayr, Margaret Mead, N. Tinbergen and A. S. Romer.

J. B. CRAGG

The Mango. By L. B. SINGH. Pp. 438. London: Leonard Hill Books Ltd. 1960. 84s.

This book is the first to appear of a new series entitled 'World Crops Books' which is intended eventually to cover every major crop, beginning with those on which little has been published in monographic form. It is an impressive sample, with many good features promising that the series will be useful. The author, who is Director of the Horticultural Research Station at Saharanpur, U.P., India, knows his subject and has spared no pains to produce a comprehensive treatment of it along the three main lines of botany, cultivation and utilization. There is a good bibliography. The illustrations are lavish and clear, the format is pleasant, and the proof correcting has been careful.

In one respect the book may not be, as the publishers claim, typical of those that will follow. While there is no doubt of the importance of the mango as the most abundantly produced tropical fruit, the amount of critical research that has been done on it is relatively slender. Consequently the author has not met the common difficulty of crop monographers in having to

compress his material. On the contrary, he uses space rather luxuriously. For example, there is a 73-page chapter on propagation, beautifully illustrated, but elementary and more suitable for a general horticultural text-book. Doubtless it is better to have too much than too little, but in the reviewer's opinion a rather less expensive volume would have done justice to present knowledge of the mango.

Some other volumes in preparation will have more to cover, and on the evidence of the sample the series is to be very warmly welcomed.

E. E. CHEESMAN

The Balkan Lake Ohrid and its Living World. By SINIŠA STANKOVIĆ. Pp. 357. Den Haag: Junk. 1960. 35 Dutch florins.

Lake Ohrid, on the borders of Yugoslavia and Albania, 348 km.² in extent, greatest depth 286 m., has long been noted for many endemic species, due to its great age, it being one of the few European lakes that was in existence before the Ice Age. The first third of the book is devoted to physiography and geology, physical factors, especially temperature, and to chemical conditions in the lake. Lists of plankton organisms are given and the distribution in time and depth of the plants is described with a rather brief discussion about the factors that could be responsible. The communities of the bottom have not been defined, which is hardly surprising as so many of the species are peculiar to the lake and the basic taxonomy has not yet been done. Up to this point the book is a limnological study along conventional lines and, as such, will not be of great interest except to limnologists. The rest will have a wider appeal. Stanković recognizes three groups: (1) Relicts. Species which are known from, or have close relatives in, a few other places or fossil deposits, or which have no close relatives anywhere are reckoned to be survivors of a fauna that has been wiped out by climatic or other changes over most of a range that was formerly much greater. (2) Immigrants. These are the common and widely distributed species. (3) Species that evolved in the lake. There are seventeen species of *Candona*, an ostracod, each with a distinct habitat, some confined to comparatively small areas of the lake, thirteen similar species of *Neodendrocoelum*, a flatworm, and smaller numbers in quite a lot of other genera. The school which holds that speciation cannot take place without complete isolation would say that these species have all originated outside and come in at different times; the shape of the basin is such that a drop in level could never have separated several lakes. Stanković, however, is convinced that there has not been anything beyond what he calls an ecological and microgeographical isolation. It is easy to see how a new invader, finding conditions suitable, might have colonized the littoral and then pushed on into the sublittoral and profundal, but less easy to see how isolation in these zones or in particular parts of the lake sufficient for the evolution of a species in each could have come about. One necessity would be some change reducing population so that there was never a surplus to wander into neighbouring biotopes. Possibly climatic factors could do this or the arrival of a new predator. Perhaps this will happen one day and its effect can be duly documented. Until then it will remain a theoretical argument and my guess is that both sides will maintain their positions.

It is obvious that no American or Briton has been consulted about the language. The word 'repartition', used throughout where we would use the admittedly rather unsatisfactory 'distribution', will puzzle readers unfamiliar with French, and the German conjunction 'resp.' is not one that we want or need in our language, but there are few places where the meaning is not clear. Rather than criticize we should express appreciation that we can now read in English this well-produced book summarizing the extensive work of Stanković and his fellow countrymen as well as much written in a great diversity of European languages.

T. T. MACAN

An Introduction to the Biology of Micro-organisms. By L. E. HAWKER, A. H. LINTON, B. F. FOLKES and M. J. CARLILE. Pp. 452, 46 figures, 40 plates. London: Edward Arnold. 1960 35s.

This introduction is divided into three sections of about equal length; on microbial morphology and life-cycles, on physiology and metabolism and on ecology. The first section deals with the

major groups of micro-organisms in turn, describing their habits, structure, life-cycles and modes of reproduction, variation and genetics. For each group a useful outline of taxonomic classification is given last.

The subject-matter of the section on physiology and metabolism is considered from first principles and covers a very wide field with remarkable economy of space: chapters 6 and 7, for example, embracing everything from simple chemical analysis of a micro-organism to a discussion of the mechanisms concerned in the detailed integration and regulation of metabolism. Students without some biochemical background would find these chapters obscure. Introductions should provide orientation for those already familiar with part of the subject and this section is particularly useful from this point of view, difficult as it may be for the novice.

The ecological section consists of a broad discussion of the microbiology of soil, air and water, and microbes in relation to plants, animals, food and fermentation. The inclusion of all this under the heading of ecology would seem to be a matter of convenience; much more than ecology is covered, and this part of the book relies heavily, and properly, on the first sections.

Other introductory works on general microbiology have appeared in recent years but this seems to me to be the most successful in maintaining balance between the different aspects of the subject. Profiting from the errors of others the authors have been able to produce a balanced work which includes much within a small compass. This has been done by economical and logical arrangement, avoiding repetition and by wholly eschewing the rambling history of this subject (Pasteur only gets a passing mention), by sensibly restricting the bibliography to general articles and by reducing discussion of technique in the text to essentials. An appendix has been added describing some techniques used in the study of micro-organisms. This is too sketchy to be of much use and would better have been left out (a modern handbook on general methods would be a useful companion to this work).

The book is written clearly in a style which maintains the reader's interest. Errors are few and the format and the numerous illustrations excellent. The subject-matter index is adequate and the text is liberally provided with useful cross-references. This book can be highly recommended to the growing number of teachers and students in universities and technical colleges who are interested in microbiology as a discipline in its own right and not an offshoot of botany, medicine or biochemistry.

P. S. NUTMAN

The Chemistry of Nucleic Acids. By D. O. JORDAN. Pp. 358. London: Butterworths. 1960. 60s.

There are two sound practical principles of biological logic: one should not assume more than one has to, and one should recognize the logical consequences of what has been assumed. The first principle should keep people from assuming that a chemist's interest in purity is shared by other organisms so that they turn out identical replicates of molecules if approximate replicates will do. The second leads to the conclusion that, if even a fraction of what is generally assumed about the 'information' storing and transferring capacity of nucleic acids is true, preparations of these acids must be vastly complex mixtures. Critical people have therefore regarded the past 20 years of increasingly naive writing on nucleic acids with dismay. The tide now seems to be turning. Prof. Jordan starts off with an emphatic chapter on the heterogeneity of nucleic acids instead of putting the customary apologetic whisper about the facts in a footnote. All preparations that have been studied carefully have been heterogeneous. This does not make them any the less interesting—only more credible. Also, it establishes a basis for profitable thought on what it is about them that leads to their being so often associated with reproductive and synthetic processes.

Having made the point that statements about nucleic acid preparations are probably statistical statements about categories rather than definite statements about entities, the book goes on to deal with the chemistry of nucleosides and nucleotides, with methods for separating complex mixtures, with the physical chemistry of isolated nucleic acids, and with the bearing that all these things have on nucleic acid structure. The tone throughout is admirably critical, difficulties are not glossed over, and an unusually serious attempt is made to distinguish observation from the garnish of interpretation. The garnish is unnecessary, for the facts themselves are exciting

enough. Thirty years ago one was amazed to read, in the preface to the best monograph on nucleic acids then available (Walter Jones, 1914), that they were 'possibly the best understood field of Physiological Chemistry'. The claim would now be more plausible.

I have only two criticisms of this excellent book. The author should be a little more consistent in specifying the source of enzymes. Tradition more or less sanctions the use of unqualified ribonuclease for the enzyme from pig pancreas, but it is wise to state this whenever there could be doubt. And he introduces too many odd and undefined words—gegenion, sonicate, pectone-mic, paranemic, etc.—for a book that should be read by a fairly broad group of scientists.

N. W. PIRIE

The Structure and Function of Muscle. In three volumes—edited by G. H. BOURNE. Volume 1, *Structure*. May 1960, 472 pp., illus. £5. os. od. Volume 2, *Biochemistry and Physiology*. August 1960, 593 pp., illus. £5. 18s. od. Volume 3, *Pharmacology and Disease*. October 1960, 482 pp., illus. £5. 7s. od. New York and London: Academic Press.

Under the editorship of G. H. Bourne the Academic Press has assembled three weighty volumes containing forty-seven articles on the present state of our knowledge of the Structure, Biochemistry, Physiology of Pharmacology, and Disease of Muscle. A distinguished assembly of contributors has been recruited for this formidable task. It would be invidious to single out any of the forty-five authors and it may suffice to say that among them are quite a number of leading pioneer workers in the field.

The gross and molecular structure, microanatomy, development, biochemistry, biophysics, physiology, pathology, and pharmacology of skeletal muscle and cardiac muscle are dealt with. The standard of illustrations is high, especially also of the numerous electron-micrographs, and the author and subject indexes are carefully compiled. In a subject in which the frontiers of knowledge are advancing rapidly, it is natural that some of the contributions are or may rapidly become dated. In some articles, the authors have perhaps not been courageous enough to omit the account of controversies of past decades which, in the light of the achievements gained with the most recent techniques, have become less meaningful, except for historical purposes. There is no doubt, however, about the timeliness of such a book which is indispensable for workers in the field and provides encyclopaedic information to the general biologist.

O. LOWENSTEIN

Planzenpathogene Bakterien. By C. STAPP. Pp. 259, 100 illus. Berlin: Paul Parey. 1958. DM. 32.

The indifferent treatment of bacterial plant pathogens in most general works on plant pathology is regrettable but probably inevitable so long as it continues to be regarded as a specialist field. Fortunately the specialists are now committing themselves to print. This latest book, by Dr Carl Stapp, is the culmination of a long career devoted to the study of these organisms. It has a special significance in so far as it marks the end of one era and the beginning of a new one. Interest in this field is now growing so rapidly that no great insight is required to predict important advances in our knowledge of the natural affinities and ecology of the bacterial plant pathogens in the next few years.

The idea of this book came to Dr Stapp after completing his account in *Sorauer*, and its object was to fulfil the need of German students for a relatively cheap text book embracing both the theory and practice of plant bacteriology. The first 67 of its 259 pages cover technique, and describe general culture methods, special media, staining procedures, biochemical tests for differentiating species, serological methods and bacteriophage typing. The latter receives little more than passing mention, but the rest is admirably done, often in considerable detail.

The main part of the work describes twenty-four species of bacteria and the diseases they cause, and includes *Agrobacterium tumefaciens*, five species of *Corynebacterium*, ten of *Pseudomonas*, five of *Xanthomonas* and two of *Erwinia*. This nomenclature is as used by the author and, as

things stand at present, strictly orthodox. The selected species are said to be of special interest to mid-Europe and include *Erwinia amylovora*, a somewhat prophetic choice considering that recent events in England must have been unknown to the author at the time. The exclusion of all tropical species seems excessively parochial but presumably the author knows what he is doing. Pathogens are arranged alphabetically. Under each are described, according to the time-honoured formula, its main distinguishing characteristics, disease symptoms, mode of infection and spread, resistant varieties, host range, geographical distribution and control methods. Each account concludes with a fairly comprehensive list of references (57 for *Agrobacterium tumefaciens*), with a distinctly cosmopolitan flavour. This alone almost makes the book worth having. There is an excellent index with species listed under both generic and specific names, suggesting that Dr Stapp is as unsure about the durability of the present generic groupings as every one else is. The book is copiously illustrated and excellently produced. It contains much of value to those interested in this particular branch of plant pathology, and anybody reasonably proficient in German is strongly urged to buy it. Those who are not ought to consider it as another good reason for learning the language.

J. E. CROSSE

Physical Chemistry for Students of Pharmacy and Biology. 2nd ed. By S. C. WALLWORK. Pp. 354. London: Longmans, Green and Co. Ltd. 1960. 18s.

This is a straightforward account of elementary physical chemistry. No attempt is made to discuss biological applications and very few of the examples given to illustrate principles are biological in character. This is also true of the numerical problems at the ends of chapters and of the series of ten practical experiments which are integrated with the text. The chapters follow a conventional sequence of the kind found in most physical chemistry texts. There is an introductory chapter on the mathematics necessary for understanding the rest of the book, and this includes sections on graphs, logarithms, exponentials and elementary calculus. A student who worked conscientiously through this book should acquire a good background in the subject necessary for the understanding of physiological phenomena. Those who knew the first edition will be glad to learn that in this revision thermodynamic ideas have been extensively introduced, the section on radioactivity has been developed, and there is a treatment of elementary wave-mechanic theory. The book is inexpensive, being paper-covered. There is a good index.

D. W. WOOD

The Control of Growth and Form. A Study of the Epidermal Cell in an Insect. By V. B. WIGGLESWORTH. Pp. vi+140. Cornell University Press. London: Oxford University Press. 1960. 21s.

Few biological investigators seem able to realize the full implications of their work, and they are seldom able to show how their discoveries may illuminate their subject more widely. Prof. Wigglesworth has this unusual gift. In this small book, based on six 'Messenger' lectures given at Cornell University in 1958, there is a clear account of his own work, done over the last 30 years, on the blood-sucking bug *Rhodnius prolixus*. Work by others, using other insects, is included, where it confirms or extends Wigglesworth's own studies, but this is mainly a book about this one insect, *Rhodnius*, and for the most part about one tissue, the epidermis of that insect. The structure and function of the epidermal cell is described, as is the way it is affected by hormones. This could have been simply a specialized account, only of interest to advanced students of insect physiology. Instead Prof. Wigglesworth moves logically from the particular to the general, and his book gives an entirely new picture of the whole question of growth and form in insects, and in effect, in the whole animal kingdom.

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